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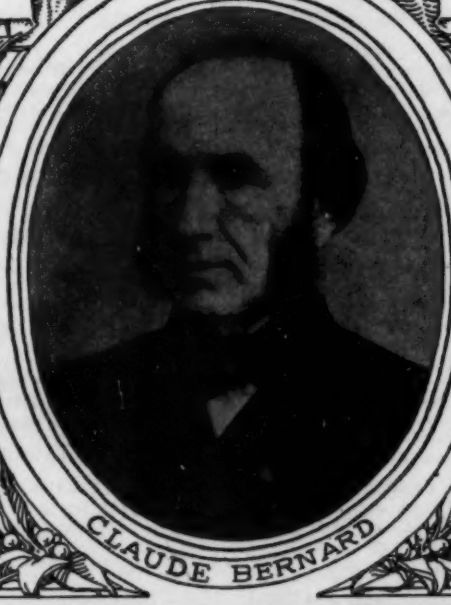

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Woodland Avenue and Thirty-sixth Street, Philadelphia, Pa.

THE EFFECT OF FERRIC CHLORIDE ON THE UTILIZATION OF CALCIUM AND PHOSPHORUS IN THE ANIMAL BODY

PEGGY REHM[1] AND JET C. WINTERS
Department of Home Economics, University of Texas, Austin

(Received for publication October 16, 1939)

There have been only a limited number of investigations on the problem of the effect of iron salts on the utilization of calcium and phosphorus. Waltner ('27) showed that adding 2% of reduced iron to McCollum's stock diet produced rickets in rats in 4 weeks. The blood serum of the rachitic rats showed low phosphorus but normal calcium, a condition similar to that found in human rickets. Cox, Dodds, Wigman and Murphy ('31), using guinea pigs and rabbits as experimental animals, showed that aluminum or ferric salts added to rations in such amounts that the aluminum or ferric ion was in excess of the phosphorus ion brought about drastic lowering of the bone ash and blood phosphorus. These investigators suggest that the reduction was due to the precipitation of alimentary phosphorus as unabsorbable ferric or aluminum phosphate. Using rats and adding ferric chloride to a non-rachitogenic diet, Brock and Diamond ('34) produced rickets of about the same degree of severity as is produced on the Steenbock rachitogenic diet. By substituting ammonium chloride for ferric chloride and finding that no rickets occurred, they showed that the ferric rather than the chloride ion was the causal factor. Deobald and Elvehjem ('35), working with chicks, demonstrated that the addition of large amounts of ferric citrate or aluminum sulfate to a diet which had been shown to be adequate for normal growth and bone formation brought about severe rickets in 1 or 2 weeks. Bone ash was reduced

[1] Died January 28, 1940.

THE JOURNAL OF NUTRITION, VOL. 19, NO. 3
MARCH, 1940

almost 50% and the phosphorus in the blood serum was markedly lowered. These investigators suggest a possible danger from the use of large doses of iron in the treatment of hypochromic anemia. Day and Stein ('38) reasoned that if the addition of iron affected phosphorus metabolism, the addition of phosphorus ought to affect iron metabolism. They studied the effect upon hematopoiesis of different levels of calcium, phosphorus, iron and vitamin D in the diet. On diets low in calcium or iron, or high in phosphorus, rats developed anemia and polycythemia. Adding large amounts of iron chloride or calcium carbonate prevented the development of the anemia, but ferric phosphate and calcium phosphate were ineffective. The explanation offered for these results is that phosphorus combines with iron and interferes with its assimilation, but that, if sufficient calcium is present to combine with the phosphorus, the iron is free for assimilation. It is in this sense, Day and Stein think, that calcium is "a sparer of iron."

Two experiments have been reported that are not in line with the conclusions of Day and Stein. Kletzien ('38) reported that various calcium salts interfered with, rather than aided, storage of iron in young rats and that rats receiving phosphoric acid utilized more iron than controls receiving an equivalent amount of phosphorus as tricalcium phosphate. Shelling and Josephs ('34) also reported that calcium hindered iron utilization in rats. Further investigation concerning the metabolism of calcium, phosphorus and iron seems timely. The present experiments offer evidence of the detrimental effect of iron salts when added to an adequate synthetic diet under conditions of controlled food intake.

EXPERIMENTAL

In the first experiment two groups of rats, properly matched as to age, sex and weight, were used. One group was fed a standard, artificial diet, and the other the same diet supplemented with enough ferric chloride to combine with one-half the amount of phosphorus present in the ration. A group was

started in which enough ferric chloride was added to combine with all the phosphorus, but these animals developed a severe anorexia and lost weight so rapidly that it was impossible to use them for experimental purposes. The rats used were of good nutritional stock. They were placed on the diet at 22 days of age and all animals used weighed between 40 and 45 gm. at this time. Each group consisted of six males and six females. The animals were placed in separate galvanized iron cages with raised bottoms to prevent coprophagy.

The diet used consisted of:

	Per cent
Casein, unpurified	20
Cornstarch	56
Butterfat	8
Salt mixture (Osborne and Mendel)	4
Yeast, dried	10
Cod liver oil	2

Food intake for the two groups was kept the same by using the animals on the supplemented diet as controls since they had poorer appetites than those on the unsupplemented ration. Rats were weighed at 4-day intervals and adjustments in the quantity of food given were made at the weighing periods. Calculations at the end of the period showed that difference in food intake amounted to less than 0.5 gm. per day per rat. At the end of 3 weeks four animals, two from each group, were killed and analyzed for total ash, calcium and phosphorus. Results showed that a definitely lower ash content for the experimental rats had already developed and it was decided to terminate the experiment at the end of 1 month. At this time the rest of the animals were killed with ether, the digestive tracts removed and each animal analyzed separately for total ash, calcium and phosphorus. The percentages of total ash, calcium and phosphorus were then calculated on net body weight. Ashing was done in weighed silica dishes; a modification of McCrudden's method was used in determining calcium and the method of The Association of Official Agricultural Chemists for phosphorus.

RESULTS

Table 1 gives the data obtained. This table shows that in spite of careful control of food intake, rats on the unsupplemented diet made greater weight gains than did those on the supplemented ration. This greater growth, in the case of the females, was made in spite of a lower food intake.

Total ash. As will be seen from table 1 the bodies of female rats kept on the synthetic diet supplemented with ferric chloride contained on the average about 0.9 gm. less total ash than did the bodies of female rats on the unsupplemented diet; the difference in the case of male rats amounted to 1.2 gm. This involves reductions of 20.0% and 25.5%, respectively. On the other hand, the percentage of total ash, based on net body weight, varied surprisingly little in the two groups of animals. This lack of variation is partly explained by the greater weight of the animals on the unsupplemented diet, but it should be remembered that this greater weight was attained without increase in mineral intake and would therefore indicate greater utilization of minerals. Variation in the amount of total ash in the individual animals of the same sex and on the same diet was not large and was not correlated directly with body weight.

Calcium. Reference to table 1 shows that as a result of the addition of ferric chloride there was a decrease in the calcium content of 0.29 gm. in bodies of the females and 0.36 gm. in the bodies of males or 23.9 and 28.0%, respectively. The average calcium, based on net body weight, in the bodies of female rats on the unsupplemented diet was 0.96%, and on the supplemented diet was 0.84%; for males, it was 0.91% and 0.81%, respectively. Individual variations in the calcium content of the animals in each group were small.

The decreases obtained in this experiment may be compared with those obtained by Sherman and Booher ('31) in an experiment in which the calcium content of the diet was varied from 0.16 to 0.32%. Sixty-day-old male rats on a diet containing 0.16% calcium had a body content of 0.7%, while those on a diet containing 0.32% calcium had a body content

TABLE 1

The effect of supplementing the diet with ferric chloride for a 30-day period on net body weight of rats, and total body ash, calcium and phosphorus

	RAT NO.	UNSUPPLEMENTED DIET							SUPPLEMENTED DIET						
		Net body weight	Total body ash		Total body calcium		Total body phosphorus		Net body weight	Total body ash		Total body calcium		Total body phosphorus	
		gm.	*gm.*	%	*gm.*	%	*gm.*	%	*gm.*	*gm.*	%	*gm.*	%	*gm.*	%
Females	1	133	4.412	3.61	1.209	0.91	0.818	0.62	—	—	—	—	—	—	—
	2	122	4.542	3.41	1.232	1.00	0.818	0.67	—	—	—	—	—	—	—
	3	124	4.524	3.64	1.226	0.99	0.804	0.65	—	—	—	—	—	—	—
	4	121	4.385	3.62	1.218	1.00	0.810	0.67	—	—	—	—	—	—	—
	5	130	4.379	3.36	1.186	0.91	0.786	0.61	—	—	—	—	—	—	—
	11	—	—	—	—	—	—	—	110	3.632	3.30	0.946	0.86	0.658	0.60
	12	—	—	—	—	—	—	—	99	3.236	3.26	0.870	0.88	0.584	0.60
	13	—	—	—	—	—	—	—	118	3.634	3.15	0.937	0.81	0.666	0.58
	14	—	—	—	—	—	—	—	109	3.485	3.19	0.911	0.84	0.636	0.58
	15	—	—	—	—	—	—	—	120	3.786	3.15	0.955	0.80	0.684	0.57
Average		126	4.448	3.53	1.214	0.96	0.807	0.64	111	3.555	3.21	0.924	0.84	0.646	0.59
Decrease from unsupplemented diet in per cent:										20.0	0.32	23.9	0.12	20.0	0.05
Males	6	141	4.756	3.37	1.278	0.91	0.866	0.61	—	—	—	—	—	—	—
	7	141	4.811	3.41	1.276	0.91	0.877	0.62	—	—	—	—	—	—	—
	8	143	4.803	3.36	—[1]	—[1]	0.869	0.61	—	—	—	—	—	—	—
	9	141	4.676	3.31	1.286	0.91	0.856	0.61	—	—	—	—	—	—	—
	10	145	4.824	3.33	1.293	0.89	0.874	0.60	—	—	—	—	—	—	—
	16	—	—	—	—	—	—	—	110	3.482	3.16	0.897	0.81	0.629	0.57
	17	—	—	—	—	—	—	—	117	3.673	3.08	0.967	0.83	0.677	0.58
	18	—	—	—	—	—	—	—	116	3.636	3.13	0.937	0.81	0.652	0.56
	19	—	—	—	—	—	—	—	107	3.239	3.02	0.845	0.79	0.585	0.55
	20	—	—	—	—	—	—	—	120	3.747	3.12	0.968	0.81	0.696	0.58
Average		142	4.774	3.36	1.283	0.91	0.868	0.61	114	3.555	3.10	0.923	0.81	0.648	0.57
Decrease from unsupplemented diet in per cent:										25.5	0.26	28.0	0.10	25.3	0.04

[1] Material lost.

of 0.87%. The difference of 0.17% was considerably larger than the difference of 0.10% obtained in the present experiment by adding ferric chloride to the diet. However, with female rats our difference of 0.12% was somewhat closer to the 0.16% obtained by Sherman and Booher.

Phosphorus. Data given in table 1 show that there was a decrease in phosphorus content of 0.16 gm. and 0.22 gm. in the bodies of female and male rats, respectively, as a result of the addition of ferric chloride to the diet. The corresponding percentage decreases were 20.0 and 25.3. The percentages of phosphorus in the bodies of female rats on the unsupplemented and the supplemented diets were 0.64 and 0.59, respectively. Corresponding figures for males were 0.61 and 0.57. The decreases in the percentage of phosphorus were not as striking as the decreases in the percentage of calcium. Just why calcium metabolism should be more drastically affected than phosphorus metabolism is a matter of conjecture. As was the case with calcium, individual variations in phosphorus content within each group were small.

In regard to total ash, calcium, and phosphorus, it may be pointed out that differences in the net body weight of animals in the two groups tended to minimize the detrimental effect of ferric chloride on calcium and phosphorus metabolism when expressed in percentage of net body weight; that is, the percentage decreases were quite small. Because of the controlled food intake, it is thought that differences in total ash, calcium, and phosphorus represent a truer picture than percentage differences. On the same calcium and phosphorus intake, much smaller amounts of these minerals were deposited in bodies of animals whose diet was supplemented by ferric chloride than in the bodies of animals whose diet was not so supplemented. It is probable that the interference with calcium and phosphorus metabolism was a causative factor in the marked anorexia and consequent poor growth of the animals on the ferric chloride supplement.

Effect of reducing cod liver oil. Because of the influence of cod liver oil on calcium and phosphorus metabolism, we

thought that it would be interesting to repeat the experiment, using smaller amounts of cod liver oil in the diet. It was also decided to adopt the pair-mate method in order to control the food intake even more accurately. In this method two animals of the same age, sex and weight are selected at the beginning of the experiment as pair-mates. The food intake for each day is carefully computed, and the animal on the smaller intake acts as control in determining the amount of food allowed his pair-mate. In this case it was, of course, the animals on the supplemented diet which acted as controls for those on the unsupplemented diet.

Four pair-mates, two on the supplemented and two on the unsupplemented diet, were studied over a period of 30 days;

TABLE 2

Net body weight of pair-mate rats

RAT	UNSUPPLEMENTED DIET NET BODY WEIGHT	RAT	SUPPLEMENTED DIET NET BODY WEIGHT
	gm.		*gm.*
2	135	8	104
3	130	9	94
4	126	10	98
5	130	11	101

the diet, in this case, contained only one-half the amount of cod liver oil as in the previous experiment. Net body weights are given in table 2. Again the rats on the unsupplemented diet made better weight gains than those on the supplemented.

Two animals on the unsupplemented diet were ashed together in the same silica dish, care being taken to select those of similar gains in weight and having similar food-intakes. The same procedure was followed with their pair-mates. Results are given in table 3.

In the previous experiment the average amounts of total ash, calcium, and phosphorus in the bodies of male rats on the unsupplemented diet were 4.774 gm., 1.283 gm., and

0.868 gm., respectively. When the cod liver oil was reduced by one-half, comparable amounts were 3.996 gm., 1.057 gm., and 0.745 gm. (average of nos. 2, 3, 4 and 5 in table 3). Thus, it will be seen that reducing the cod liver oil decreased the amounts of total ash, calcium and phosphorus in the body. This would be expected, since cod liver oil is known to improve retention of calcium and phosphorus. The percentage differences, however, between the animals fed the supplemented and unsupplemented diet were smaller when the cod liver oil was lowered. In the previous experiment

TABLE 3

Effect of ferric chloride on total ash, calcium, and phosphorus in bodies of pair-mate male rats

RATS		TOTAL ASH	CALCIUM	PHOSPHORUS
		gm.	gm.	gm.
Controls	2 and 3	8.010	2.119	1.498
Pair-mates	8 and 9	6.499	1.683	1.183
Decrease		1.511	0.437	0.315
Per cent decrease		18.9	20.6	21.0
Controls	4 and 5	7.972	2.109	1.483
Pair-mates	10 and 11	6.362	1.704	1.198
Decrease		1.610	0.405	0.285
Per cent decrease		20.2	19.2	19.2

the average percentage differences in total ash, calcium and phosphorus in the bodies of male rats were 25.5, 28.0, and 25.3, respectively. When the cod liver oil was reduced by one-half, comparable percentages were 19.5, 19.9, and 20.6 (average of decreases in table 3). This would indicate that the effect of adding ferric chloride is less drastic when small amounts of cod liver oil are supplied in the diet. From the standpoint of iron utilization these results indicate that iron might be better utilized when small rather than large amounts of cod liver oil are added to the diet. Less of the phosphorus is evidently bound as ferric phosphate; therefore more iron should be available.

SUMMARY AND CONCLUSION

A comparison of the amounts of total ash, calcium, and phosphorus in the bodies of animals on an unsupplemented diet with the amounts in bodies of animals on a diet supplemented with enough ferric chloride to combine with one-half the phosphorus of the diet was made, and it was shown that the addition of ferric chloride resulted in a considerable reduction in the amounts of total ash, calcium, and phosphorus at the end of 30 days. Similar experiments with like results have been reported in the literature, but in no other study has the food intake of the two groups of animals been equalized so that mineral intake of both groups was the same. In the present investigation the food intake of all animals has been kept approximately the same. In spite of this equalized food intake, animals on the unsupplemented diet gained more weight, and had larger amounts of calcium, phosphorus, and total ash deposited in their bodies at the end of the experimental period than animals with the ferric chloride supplement.

Although the analysis of each animal was carried out separately, discussion of results was based on group averages. Sex differences were not considered to be of such magnitude as to warrant separate discussion. Comparisons were made of percentage and total calcium, phosphorus, and ash in the bodies of both groups of animals. It has been pointed out that differences in net body weight of animals in the two groups tend to minimize the detrimental effect of ferric chloride on calcium and phosphorus metabolism, when expressed in percentage of body weight. Because of controlled food intake, it was thought that differences in total ash, calcium, and phosphorus represent a truer picture than percentage differences in arriving at a conclusion as to the effect of iron on calcium and phosphorus metabolism. Results of this experiment indicate that ferric chloride has a detrimental effect on calcium and phosphorus metabolism.

When the amount of cod liver oil in the diet was reduced by 50% the addition of ferric chloride resulted in a less drastic lowering of body calcium and phosphorus.

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INVESTIGATION OF THE VITAMIN C CONTENT OF FLORIDA FRUITS AND VEGETABLES

I. EFFECTS OF MATURATION AND OF COLD STORAGE ON THE VITAMIN C POTENCY OF ORANGES AND GRAPEFRUIT [1]

R. B. FRENCH AND O. D. ABBOTT

Division of Biochemistry, Department of Home Economics, Florida Agricultural Experiment Station, Gainesville

FIVE FIGURES

(Received for publication November 24, 1939)

The development of quick chemical methods for the determination of vitamin C has stimulated the publication of considerable work dealing with its natural distribution. Citrus fruits, long known as an important source of vitamin C in the human dietary, have received their share of attention. Many investigators (Harris and Ray, '33; Bacharach, Cook and Smith, '34; Guerrant, Rasmussen and Dutcher, '35; Daniel, Kennedy and Munsell, '36) have published results indicating considerable variability in the vitamin C content of citrus fruits.

This paper is a report on the effect of maturation, location of grove, and cold storage on the vitamin C concentration of oranges. Some studies of the effect of storage on the vitamin C potency of grapefruit are also included. Beacham and Bonney ('37) published estimations of the vitamin C content of Florida citrus fruits as affected by such factors as variety and root stock differences, picking dates and geographic location, but did not attempt to draw any conclusions from their variable and extensive data. Their samples, like ours, were

[1] Published with approval of the director of the Florida Agricultural Experiment Station.

gathered during the season of 1936 and 1937 and in one case a comparison of their results with ours from the same grove is possible.

METHODS

Four dozen or more fruit were picked and within a day taken to the laboratory for analysis. The fruit was then graded according to size and color. Obviously off-size, whether small or large, or off-color fruit was either discarded or made up in separate samples. A few determinations on off-color fruit are reported; the number of fruit in these samples ranged from three up to the minimum requirement of twenty-four for a satisfactory sample.

The fruit was weighed, peeled (using a glass knife), and the juice expressed through cheese-cloth in a porcelain-block press, care being taken that the sample at no time came in contact with metals. The juice volume represented the easily expressible juice. Weights of the fruit and skin and of the volume of juice were recorded, and acid and vitamin C determinations were made on the juice.

Total acidity was determined using phenolphthalein as an indicator. The vitamin C was determined by titrating a milliliter of juice, to which a milliliter of 10% trichloracetic acid had been added (Birch, Harris and Ray, '33) with Tillmans' indicator (0.2% aqueous solution of sodium 2,6,dichlorobenzenone-indophenol, Eastman) (Tillmans, '30). The indicator solution was standardized against ascorbic acid.[2] One milliliter of the purified indicator was equivalent to approximately 1 mg. of ascorbic acid.

EXPERIMENTAL

Maturity. In the grove where the fruit was obtained, the major bloom occurred in January and February, and fruits set from these blossoms attained considerable size in October. Sampling, therefore, began the middle of October, and continued thereafter at 14-day intervals until June, 1937. The

[2] Cebione from the Merck Company.

fruits collected came from individual trees and were of three varieties, Parson Brown (early ripening), Pineapple (mid-season) and Valencia (late ripening). The Parson Brown oranges in this grove were picked for market in November, and the Pineapple and Valencia during January and March, respectively.

The data on the effect of maturation on both acid and vitamin C content of Parson Brown, Pineapple and Valencia oranges are presented in spot diagrams 1, 2 and 3. For purposes of comparison, the data of Beacham and Bonney ('37) are also given.

There was a decrease of acidity in all the oranges as maturation advanced, the rate being about the same for the Parson Brown and Pineapple, and a little greater for the later-ripening Valencia. The decrease continued without apparent change in rate as long as the oranges remained on the tree.

The distribution of points on the graphs shows that the vitamin C concentration during the 6-month period of observation remained surprisingly constant. Such tendencies as might be suggested are masked by individual variation, and more data would be needed to establish them.

Unripe green oranges taken from different parts of the tree—top, bottom, inside and outside branches—showed little variation in either acid or vitamin C content, but after general crop yellowing both the Parson Brown and Pineapple oranges that did not color well produced juice that was judged of lower quality and was usually lower in vitamin C. However, the poorly-colored Valencias might be either higher or lower in content of acid and vitamin C, the difference in this case probably being due to the difficulty in separating the "off" colored fruits from those showing a normal "greening up." The variability in vitamin C concentration among oranges obtained from different sources may be considerable (table 1); among like-appearing oranges from the same tree it is very much less (figs. 1, 2 and 3). The parallelism between our data and those of Beacham and Bonney ('37; crosses in figs. 1 and 2) is noteworthy and suggests that analyses of oranges

from the same tree show a considerable degree of reproducibility; also, that analyses of oranges of the same variety from neighboring trees may or may not show equal vitamin C concentration, but that the value obtained is probably characteristic for the tree. The oranges in these and in Beacham and

TABLE 1

Concentration of vitamin C in citrus fruit grown in several localities, and the effect of storage

VARIETY	LOCATION IN FLORIDA	VITAMIN C IN MILLIGRAMS PER 100 ML.	
		As obtained	After storage 1 month at 42°F.
Pineapple orange	North	55	68
	Central	65 [1]	52
	Central	75	91
	Central	53	..
	Central	60	..
	East coast	65 [1]	53
	East coast	68	79
	East coast	70	81
Florida seedling grapefruit	Central	47	54
	Central	38	..
	Central	46	..
	Central	56	..
	East coast	44 [1]	41
	East coast	40	45
	East coast	38	48
Valencia orange	North	48	
	Central	38	
	Central	38	
	Central	36	
	Central	33	
	Central	49	
	Central	48	
	Central	50	

[1] Fruit had been processed before packing.

Bonney's experiments were obtained from the same grove but from different trees. When considered as a whole no association could be detected between the concentration of vitamin C in the oranges used in these studies and the weight, amount of peel, or volume of juice.

That there is considerable drop in vitamin C concentration at some early stage of development is shown by analyses on samples of green, young Valencias. These weighed less than 10 gm. apiece and the expressed juice gave high titration readings averaging 347 and 335 mg. of vitamin C per 100 gm. for the outer rind and the inner portion of the fruit, respectively. Such values indicate that in these minute fruit there is almost as great a total quantity of vitamin C as there is in a small mature fruit.

Location. Oranges and grapefruit were obtained from the north, central and east coast citrus regions of the state.

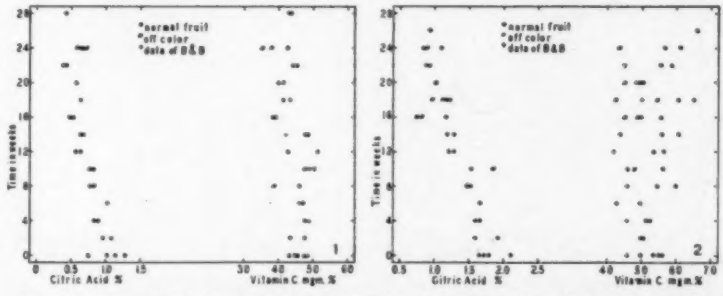

Fig. 1 Changes in citric acid and vitamin C concentration during maturation of the Parson Brown orange between October 16th and April 30th. "Data of B & B" are those of Beacham and Bonney ('37).

Fig. 2 Changes in citric acid and vitamin C concentration during maturation of the Pineapple orange between October 16th and April 2nd. For "data of B & B" see Beacham and Bonney ('37).

These were analyzed according to the procedure outlined, and the average results are given in table 1. The range of values for vitamin C seems to bear no relation to the section in which the fruit was grown. This suggests that climatic or geographical influences within the regions studied were not factors of major importance. This conclusion is supported by the data presented by Beacham and Bonney ('37).

Storage. Pineapple oranges from six different sources and Florida seedling grapefruit from four different sources were put in storage at 42°F. Samples of these fruits were removed

at frequent intervals during a period of 5 months and determinations made upon them according to the outlined procedure.

The data were gathered together in the form of spot diagrams and observations made on the interrelationships between weight, volume of juice, length of storage period, and concentrations of acid and vitamin C. Except for correlations between acid and vitamin C, and between length of storage period and both acid and vitamin C, no associations between the several factors could be noted.

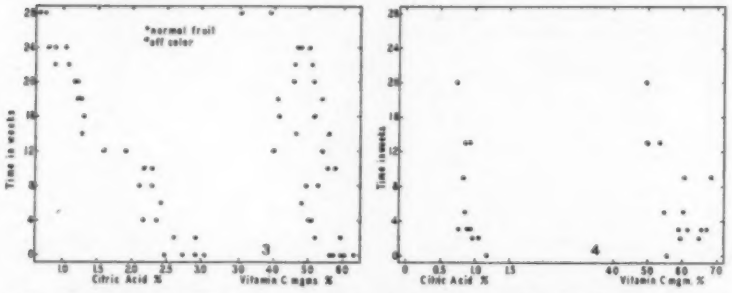

Fig. 3 Changes in citric acid and vitamin C concentration during maturation of the Valencia orange between October 16th and April 30th.

Fig. 4 Changes in citric acid and vitamin C concentration in the Pineapple orange during storage from February 2nd to June 28th.

An example of the data obtained from one crate of Pineapple oranges is given in figure 4. These were oranges picked from the Pineapple tree 16 weeks after the start of the studies of the influence of maturity. A comparison of the data of figure 2 from 16 weeks on with those of figure 4 shows that both the acid and vitamin C concentrations go through about the same changes whether the orange remains on the tree or is put in cold storage.

A small increase in vitamin C concentration after 2 and 3 weeks in storage can be noted in figure 4. This change may not in itself be significant, but seven out of the ten stored samples of oranges and grapefruit showed variable increases

in vitamin C concentration after storage for 1 month. The extent of these changes, increases up to 26% for seven samples and decreases amounting to 20% for three, is shown in table 1. Examination of the fruit that failed to increase in vitamin C content showed that (contrary to our request) it had been processed before being shipped. Processing may have interfered with normal metabolic changes.

The effect of loss of weight due to drying out of the oranges would not significantly affect these results since Stahl and Fifield ('36) have shown that oranges treated similarly will lose less than 3% of their weight in a month's time.

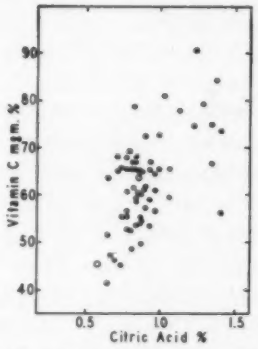

Fig. 5 The relation of citric acid content to that of vitamin C in ripe Pineapple oranges during storage at 42°F. for 5 months.

Figure 5 is a spot diagram depicting the relationship of acid and vitamin C in the Pineapple oranges for the whole storage period of 5 months. Considerably higher values for vitamin C are associated, apparently, with slightly higher acidity values. Among the individual samples that make up these data we find four cases in which there is such an association and two cases in which it is lacking. With the grapefruit, association was obtained in only one case while in three others it was lacking.

There has been a tendency to postulate an association of acid and vitamin C in citrus fruit, partly because of the

fact that, in vitro, acid tends to stabilize vitamin C solutions, and also because of other observations such as those of Lorenz ('36). The data presented in this paper do not deny the possibility of an association but they suggest that any simple association should not be very good, because during both maturation and storage of the fruit fluctuations in vitamin C concentration occur that are unaccompanied by similar changes in acid concentration.

DISCUSSION

The experimental error of a vitamin C determination is easily kept within 2%. The end point of the titration is sharp with citrus juices if the indicator is freshly prepared. Grinding the fruit pulp in a tinned meat-chopper or extracting in a steel press may cause a diminution in titration value, presumably due to contamination with iron, and for these reasons metal contacts in preparing the fruit were avoided.

How accurately the titration procedure measures the vitamin C in citrus juice is still a question. The value obtained has an accuracy at least as great as that determined by the biological procedure, since it has been shown that values secured volumetrically check reasonably well with those obtained by feeding experiments (Tillmans and Hirsch, '33; Harris and Ray, '33).

The cause of the increase noted in vitamin C content of stored fruit was investigated, but no positive conclusions were reached. Hydrolysis of the juice of fresh fruit in 10% phosphoric acid under carbon dioxide produced no change in titration value. Evidently, no ester combination of ascorbic acid is present as was suggested by Reedman and McHenry ('37) in dealing with a similar phenomenon in vegetables; nor could the presence of reversibly oxidized ascorbic acid be demonstrated by following the procedure of McHenry and Graham ('35).

Confusion over the demonstration of reversibly oxidized ascorbic acid may possibly arise in the following way. If the sample has been contaminated with iron or copper, a lowered

titration value may be obtained as noted by Barron, Barron and Klemperer ('36) and others. If the contamination occurs after the addition of acid, the value obtained will be higher if phosphoric rather than any other acid is used. Phosphoric acid tends to protect the vitamin under such conditions by precipitating the heavy metal. In either case if the contaminated samples are reduced with H_2S and titrated, a definite increase in value will be obtained. The increase will be proportional to the quantity of iron (or copper) contamination since ferrous (or cuprous) sulphide is oxidized by the indicator.

SUMMARY AND CONCLUSIONS

Differences in vitamin C content both among samples of one variety and among different varieties of citrus fruit may be very large. Individual trees tend to produce fruit the vitamin C content of which varies only within narrow limits, but these small variations are sufficient to mask any changes occurring during maturation of the fruit. Changes in vitamin C content of oranges during maturation are not large.

Variations in vitamin C content among oranges did not correlate with the different geographical locations in which they were grown.

The vitamin C concentration of oranges and grapefruit may increase during the first few weeks of cold storage; after this, the concentration of vitamin C drops off slowly.

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GREYING OF FUR AND OTHER DISTURBANCES IN SEVERAL SPECIES DUE TO A VITAMIN DEFICIENCY [1]

AGNES FAY MORGAN AND HELEN DAVISON SIMMS [2]
Department of Home Economics, University of California, Berkeley

THREE FIGURES

(Received for publication October 12, 1939)

Loss of pigmentation in the fur of rats fed diets deficient in one or more of the B vitamins has been observed by various investigators (Bakke, Aschehoug and Zbinden, '30; Gorter, '34, '35; Hartwell, '23; György, '35). Similar changes were seen in rats made anemic by the use of whole milk diets by Keil and Nelson ('31). The latter workers and Gorter ('35) stated that administration of small amounts of copper salts produced darkening of the bleached hair.

Morgan, Cook and Davison ('38) were the first to note that the active substance which prevents and cures the greying is present in the filtrate fraction of the vitamin B_2 complex, that is, remains in the water extract of yeast, liver or rice bran after repeated treatments with fuller's earth have removed vitamins B_1, B_6 and riboflavin. Lunde and Kringstad ('39) have fully confirmed this observation and further have concluded that the "rat growth" and "chick anti-dermatitis" filtrate factors of Lepkovsky, Jukes and Krause ('36) and Elvehjem and Koehn ('35) are not identical with the anti-greying factor.

[1] Some of these data were presented before the Sixth Pacific Science Congress at Berkeley, California, on July 27, 1939.

[2] Assistance in this investigation has been rendered by Works Progress Administration Official Project 665-08-3-30, Unit A-24, assigned to the University of California.

Whether the greying observed in anemic rats which is cured by copper salts is different in character from that seen in non-anemic rats lacking the anti-grey factor of the B_2 complex remains to be determined. It is possible that both copper and the anti-grey filtrate factor are necessary for maintenance of the color of fur and that deficiency in either will produce depigmentation. The whole milk diet which is used for nutritional anemia production is certainly low in the "filtrate factor" as well as in iron and copper (Jukes and Richardson, '38).

In none of the earlier observations of depigmentation has there been any correlation of the phenomenon with other changes, particularly those of senescence, which might be expected to accompany the greying of the hair. It is clear that the greying must be caused by failure of pigment formation or by destruction of pigment and that the mechanism controlling this function may be damaged. Since both the adrenal and thyroid glands have been thought to be concerned with pigment and hair growth either directly or indirectly through the sex glands, these were examined in the greyed animals.

EXPERIMENTAL METHODS

The basal diet which we have used to produce the greying consists of washed casein [3] 22, salt mixture (Hubbell, Mendel and Wakeman, '37) 2.5, Crisco 9, and sucrose 56.5 parts. The daily supplements per rat were thiamin chloride 10 micrograms, riboflavin 30 micrograms, wheat germ autolysate or eluate equivalent to 0.5 gm. wheat germ, cod liver oil 4 drops. Young rats were placed at weaning on the basal diet supplemented by the thiamin chloride and cod liver oil for 3 to 4 weeks, at the end of which time their growth had ceased. The riboflavin and wheat germ eluate (as source of vitamin B_6) were then given with prompt but usually temporary

[3] We are grateful to the Western Condensing Company of San Francisco which through the courtesy of Drs. P. D. V. Manning and E. L. R. Stokstad provided the supply of washed casein used in this investigation.

resumption of growth. After 56 to 70 days at least half of all such rats began to show the characteristic changes in the fur.

RESULTS

The greying of the fur

The greying was preceded by a change in texture of the fur which became dry and somewhat brittle, a dryness which was also noted in the fur of white rats fed the same deficient diet.

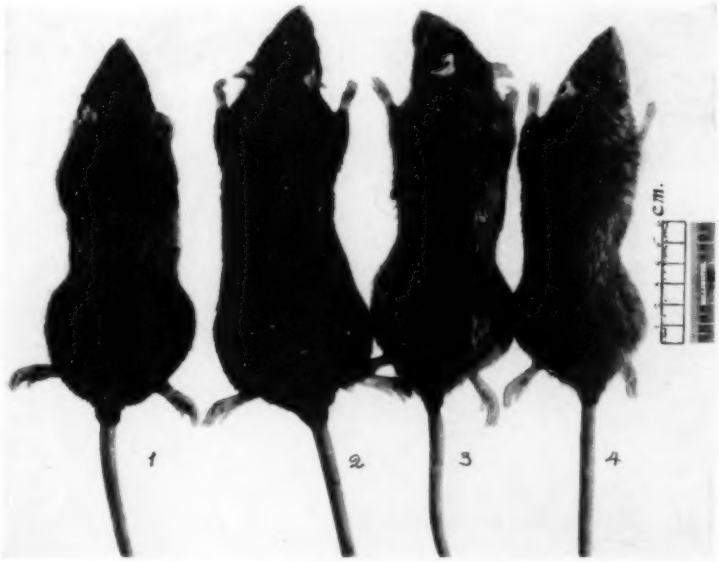

Fig. 1 Typical greying animals of the same litter. Numbers 1, 3 and 4 were fed the anti-grey vitamin-deficient diet; number 2 received the same diet plus the missing substance.

The fur then became a dull mousey brown, metallic and finally grey in color. The mousiness and greyness occurred usually in a definite bilateral symmetrical pattern. The most common pattern was that in which the top of the head and the sides of the body were greyed. Examples of greyed rats are shown in figure 1 along with a normal rat of the same litter.

Some seasonal influence has been seen in the phenomenon, as illustrated in table 1 by the rate of greying of eight groups of rats started on the diet over a period of 2 years. More rapid greying occurred in the later summer and autumn than in winter and spring but there was much variation. Perhaps the seasonal changes in rate of growth of hair may account for this since the depigmentation was found to originate in the follicle and to be observable only as the normal hair was shed. Apparently at least 30 days are required for the new hair growth to affect the color of the hair tips.

TABLE 1

Time required for greying of rat fur due to vitamin deficiency

NUMBER OF ANIMALS	DATE STARTED AFTER PRELIMINARY DEPLETION	DATE WHEN MORE THAN 50% WERE GREY	TIME REQUIRED FOR GREYING
			days
33	May 20, 1937	July 15, 1937	55
12	July 29, 1937	October 7, 1937	70
32	September 23, 1937	December 2, 1937	70
33	December 2, 1937	March 1, 1938	89
37	May 26, 1938	July 21, 1938	56
15	December 21, 1938	March 1, 1939	70
	WITHOUT DEPLETION		
18	October 12, 1938	December 21, 1938	70
35[1]	February 15, 1939	June 13, 1939	120

Total 215.

[1] Wheat germ diet used.

This was the shortest period in which complete return to normal color was produced by any curative agent. The blue black coloration of the skin of the dark areas in pied rats was also bleached in the process of greying and darkened when cures were effected.

Dermatitis

In addition to greying we have noticed in the cases of chronic insufficiency in older animals a dermatitis which is unlike that due to specific vitamin B_6 deficiency. It is manifested by groups of small lesions located behind the shoulders

and extending partially down the sides (fig. 2). The fur around these lesions is often thin, dry and discolored. No loss of weight accompanies this condition.

Among all the animals regardless of age there often developed also a different type of dermatitis similar in some respects to that in vitamin B_6 deficiency. It was characterized first by a reddening of the nose and a sloughing off of

Fig. 2 Normal and grey rats and those showing the shoulder type dermatitis of the anti-grey vitamin deficiency. Numbers 1 and 4 have shaved patches on the shoulders to show the peculiar type of eruption. This and the ulcers developed mostly in deficient rats which resisted greying.

the fur leaving the skin raw and exuding a moist brownish fluid. This sloughing gradually extends over the face, around the eyes, to the top of the head, to the skin of the abdomen and the legs above the knee and elbow joints. It is important to note that the paws are unaffected usually and that the skin is

wrinkled. Within a short time the animal loses considerable weight and death soon ensues. If, however, filtrate factor is administered, it is possible for the animal to be almost completely cured, within 4 to 12 weeks this including the growth of a new thick pelage, loss of dermatitis and return to previous weight.

Ulcers

In a few of these elderly animals there occurred a severe sloughing of the skin usually beginning first above the base of the tail. The denuded area was moist, inflamed and hemorrhagic, and reminiscent of the lazy leg ulcers observed in humans in the tropics. The slowly spreading ulcer, 2 inches in diameter on one rat, was not cured by administration of as much as 40 micrograms per day of crystalline vitamin B_6.[4] This rat had been fed the filtrate factor deficient diet for 18 months without greying of the fur or interference with normal growth. In November, 1938, the first sign of ulceration began and by March, 1939, the condition had become very severe. At this time over a period of 5 weeks a total of 1.05 mg. crystalline B_6 was injected in gradually increasing doses but without effect upon the ulcer. In May, 1939, a filtrate factor concentrate made from brewer's yeast was fed in amount equivalent to 3 to 5 gm. of yeast daily. Almost at once the ulcer began to dry, scar tissue was formed about the periphery, and by July, 1939, the damage was completely repaired. The vitamin administration was stopped in September, 1939, and 2 months later the ulcer area was once more inflamed.

Another rat, fed the deficient diet from December 6, 1937, when it was 7 weeks old, developed the same type of ulcer in December, 1938. From March 13, 1939, wheat germ oil,[5]

[4] We are indebted to Dr. S. Lepkovsky for a supply of this material.

[5] The wheat germ oil was tried because another rat with similar ulcer for 7 months had improved when fed whole wheat germ ad libitum for 2 months. Although wheat germ contains some filtrate factor it is not rich in this substance and it was necessary to determine whether the oil phase rather than the B vitamin factors of the germ had the curative effect.

5 cc. per day was fed until April 17, 1939, but without effect. The ulcer continued to spread. This rat was then treated with injections of adrenal cortex extract,[6] 180 D.U. per week, for 12 weeks but without improvement. In December, 1939, the ulcer was still raw and bleeding and the fur had developed snow-white patches, although the normal glossy black color had persisted through nearly 2 years of deficiency.

Relation of growth to greying

The growth of rats on this diet was variable but usually subnormal. In general the rats which greyed early grew less than those which resisted the greying effect. At first we were led by this coincidence to believe that the anti-grey and the "rat-growth filtrate factor" were identical but later experience has shown that this is possibly not true. Preparations which produce rapid cure of the greying may allow little increase in growth, and diets upon which excellent growth is obtained may allow early and complete greying. In our earlier experiments in which a particular brand (A) of brewer's yeast, 0.5 gm. per day, was used for positive controls greying was never seen in these animals. Later when another brand (B) of brewer's yeast was substituted, greying of well-growing rats often occurred. This was seen first in animals used for certain protein tests and also in vitamin A assays. Obviously yeast "B" was less well endowed with the anti-grey vitamin than yeast "A", although equally good growth was obtained with both.

Cures of the greying were effected promptly by preparations which promoted little growth as may be seen in figure 3. The cane molasses and alfalfa extracts induced rapid growth but only late and incomplete cure of greyness, while rice bran and liver filtrates had less effect on growth but caused prompt darkening of the fur. This was strikingly true of the rice bran preparation. The yeast filtrate produced some growth and fairly rapid darkening of the fur.

[6] The adrenal cortex extracts were kindly provided by Dr. Oliver Kamm of Parke, Davis and Company.

The "chick anti-dermatitis factor" which has been said by Jukes ('39) and by Woolley, Waisman and Elvehjem ('39) to be identical in properties with the pantothenic acid of R. J. Williams may or may not be the same as the rat growth factor or the anti-grey factor. This was tested by the following experiment.

A yeast eluate was made by extraction of yeast B (see above) with 50% ethyl alcohol, concentrated, acidified with HCl and treated with fuller's earth repeatedly. The adsorbate, after thorough washing with very dilute HCl, was eluted with dilute ammonium hydroxide, washed several times and the eluate and washings concentrated until 1 cc. was the

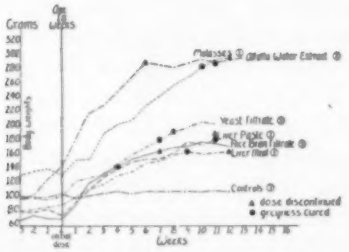

Fig. 3 Relation of anti-greying to growth responses produced by various filtrate preparations.

equivalent of 45 gm. yeast. The concentrate was made strongly alkaline with sodium hydroxide and boiled under reflux for 6½ hours, then cooled and neutralized with HCl. The resulting product, yeast fraction 325-A, contained the equivalent of 26 gm. of yeast per cubic centimeter but little pantothenic acid if this substance is destroyed by such prolonged heating in alkaline medium. This product, supplied by Dr. T. H. Jukes, in 0.1 cc. daily doses rapidly cured the greying and at the same time promoted the growth of young rats which had been in an advanced stage of the anti-grey deficiency condition. The product had however retained a mild anti-dermatitis efficacy when tested on chicks.

Pure beta-alanine, which was shown by Woolley, Waisman and Elvehjem ('39) to be part of the pantothenic acid molecule, when fed in doses of 100 to 250 micrograms daily to grey rats increased the severity of the symptoms and caused early death. This is similar to the effect of nicotinic acid.

Specificity of the anti-grey vitamin

Various other substances have been tried both as preventives and cures particularly for the greying but also for the dermatitis, and to determine the relation of the other vitamins of the B complex to these conditions. The following facts were brought to light.

Nicotinic acid or amide does not cure or prevent greyness, and in excess apparently exaggerates the dermatitis of the face and abdomen and hastens the death of the animals.

Vitamin B_1 or riboflavin in doses of 200 to 250 micrograms daily, vitamin B_6, vitamin B_6 and riboflavin together in excess, wheat germ, wheat germ oil, and copper (as $CuSO_4$) have all been found to be ineffective in curing or preventing the greying and the dermatitis.

Yeast, yeast filtrate, concentrated acid yeast eluate, liver meal, liver paste, liver filtrate, rice bran filtrate, crude cane molasses and alfalfa extract cure the greyness at varying rates of speed. Yeast and rice bran filtrates cure all types of dermatitis produced by the deficiency.

An illustrative experiment to determine the effect upon the greying by the various constituents of the vitamin B_2 complex was made upon forty-four weanling black rats from six litters. All of these animals were depleted by 4 weeks of subsistence on the usual basal diet with vitamin B_1 and cod liver oil supplements.

After 4 weeks, growth had practically ceased. The rats were then divided into six groups with daily additions to their diets for 6 weeks as follows: (1) no addition; (2) 0.5 gm. brewery yeast; (3) riboflavin, 30 γ; (4) wheat germ (acetone extracted) autolysate equivalent to $\frac{1}{2}$ gm. wheat germ as source of vitamin B_6; (5) yeast filtrate free from riboflavin and vita-

min B_6, equivalent to 1 gm. yeast; (6) riboflavin, B_6 preparation and yeast filtrate in the amounts mentioned for the preceding groups. The plan was to produce each type of double deficiency for 6 weeks, then supply one of the missing vitamins to some of the animals of each group, thus providing also single deficiencies for 6 weeks. Finally for a third 6 weeks' period there was given to some rats of each group the third missing factor. At the end of 18 weeks after depletion there were therefore a few rats showing all the possible double and single B_2 deficiencies as well as some which had been cured successively of these deficiencies.

The groups given yeast and all three of the B_2 factors, i.e., groups two and six, appeared normal at all times although group six did not grow satisfactorily. The other four groups which were deficient in either two or three of the B_2 vitamins made little or no growth but no greying of fur occurred except in the rats receiving the B_6 preparation. In the second 6 weeks' period when there were single as well as double deficiency groups greying occurred only in the animals receiving riboflavin and B_6. In the third 6 weeks' period in which some of each deficient group received one or both of the missing factors, greying again occurred only in those groups which received B_6 only, or B_6 and riboflavin. Cures were seen in each of the greying groups to which filtrate preparations were administered during either the second or third periods. At the end of the eighteenth week, of the sixteen surviving rats which had not received filtrate factor, ten were completely grey. Five of the others were suffering from B_6 deficiency and only one which received B_6 but no filtrate factor had normal pigmentation.

Double deficiencies were manifest in several of these animals. The mole-like fur and denuding appeared along with the greying in several of those which were deficient in both riboflavin and filtrate factor. The rats which were deprived of both B_6 and riboflavin, in several instances survived long enough to exhibit simultaneously fur and skin symptoms typical of both deficiencies. It was not possible usually to obtain

in the same animals the acrodynia of B_6 deficiency and the greying effect of filtrate factor deficiency. However, several animals have been observed which have developed simultaneously the triple deficiency, i.e., acrodynia of vitamin B_6 deficiency, ariboflavinosis and greying of the fur.

The damage to the adrenal glands

Histological studies of rats fed the deficient diet were made, with special attention given to the condition of the adrenals, thyroid, skin and gonads. Parallel examination was also made of litter mates on the same diet but deficient in riboflavin, or vitamin B_6 instead of filtrate factors. The chief distinctive change found was in the adrenal glands of the filtrate factor deficient animals. These microscopic studies will be reported in full elsewhere.

In animals which had greyed, the zona reticularis was degenerated, with heavy deposits of yellow pigment and connective tissue and excess vascularity.[7] These conditions were not marked in the deficient animals which had not greyed and were largely remedied in those which had been cured by administration of the anti-grey preparations.

The thyroids of the grey animals were also damaged, with no serration of colloid which stained quite uniformly instead of irregularly as in the normal thyroid. Some acini were totally filled with epithelial cells, thus indicating relative inactivity of the gland. The more advanced the greying of the fur, the greater was the abnormality of the thyroid.[8]

No such changes were seen in the adrenals and thyroids of rats fed diets deficient in riboflavin or vitamin B_6.

Loss of the elastic layer of the skin and failure of spermatogenesis were also noted in the greyed rats.

[7] A preliminary note on this phenomenon appeared in Science, vol. 89, pages 565–566, June 16, 1939.

[8] Dr. Jesse L. Carr of the Pathology Department, University of California, School of Medicine, gave us indispensable assistance in the preparation and interpretation of the tissue studies.

Treatment with adrenal cortex extract, adrenalin and thyroid extract separately or in various combinations was tried as means of producing a cure of the greying with the results shown in table 2. The adrenal cortical extract was most effective in curing the condition but thyroid was also effective. The adrenalin appeared on the whole to be of indifferent value, although a sudden and complete greying occurred in several animals 2 or 3 weeks after the adrenalin treatment was discontinued. In all cases the darkening of the hair was accomplished far more slowly with the cortin and thyroid extract than with potent filtrate preparations.

Effect on lactation[9]

In the absence of the filtrate factor or factors failure of lactation occurs invariably in rats. Adult female rats which had borne and successfully reared a first litter were placed on the experimental diet on the day of mating or on the day of littering. Good records were secured with the yeast supplement, with all three vitamin additions, that is, B_6, riboflavin and filtrate factor, poorer growth with B_6 deficient and riboflavin deficient diets but no survival of young on filtrate factor deficiency except in a few cases when the deficient diet was given only after the young were born.

Two specific lactation-controlling vitamins, one found in liver and one in yeast, have been postulated by Nakahara, Inukai and Ugami ('38). Certainly in our experiments success in lactation was possible with a diet containing the filtrate factor preparation and failure resulted on the same diet and identical supplements but without the filtrate factor or factors.

Experiments with guinea pigs

An attempt was made to produce the symptoms of the deficiency in guinea pigs. This was difficult because of the poor response of this species to synthetic diets. However,

[9] The work on lactation was done by Miss Lura M. Morse. A full report of this will appear later.

TABLE 2

Summary of curative treatment of greyed rats with substances other than members of the vitamin B complex

RAT NO.	AGE AT BEGINNING OF TREATMENT	MATERIAL USED IN THE TEST	FIRST LEVEL OF DOSAGE		SECOND LEVEL OF DOSAGE		REMARKS
			Per week	For	Per week	For	
	weeks			*weeks*		*weeks*	
192	14	Cortin	40 DU [1]	13	180 DU	10	Grey decreased. Died after 23 weeks of treatment
250	14	Cortin	40 DU	6	80–180 DU	15	Grey disappeared after 6 weeks
6110	16	Cortin	180 DU	6			Grey disappeared
254	14	Cortin Thyroid	40 DU 1–2 grains	12	180 DU 2 grains	10	Grey disappeared after 15 weeks
197	14	Cortin Thyroid	40 DU 1–2 grains	12	180 DU 2 grains	10	Grey decreased. Died after 22 weeks of treatment
204	14	Cortin Adrenalin	40 DU 1.5 cc.	1			Died
252	14	Cortin Adrenalin	40 DU 1.5 cc.	12	180 DU 1.5 cc.	9	Grey disappeared after 8 weeks, then brown color appeared
257	14	Adrenalin	1.2 cc.	8	1.5 cc.	12	Some darkening after 20 weeks, then greyed suddenly
190	14	Adrenalin	1.2 cc.	8	1.5 cc.	12	Darkened after 17 weeks, then greyed suddenly
202	14	Thyroid extract	1 grain	5	2 grains	17	Grey disappeared after 15 weeks
189	14	Thyroid extract	1 grain	2			Died
153	61	Ascorbic acid	16 mg.	11			No change
1850	25	Ascorbic acid	16 mg.	11			No change
7780	9	Ascorbic acid	16 mg.	11			Slight darkening after 10 weeks
191	14	Thyroid Adrenalin	1 grain 1.5 cc.	4	2 grains 1.5 cc.	10	Grey largely disappeared after 12 weeks, but reappeared on discontinuing the adrenalin

[1] Dog units.

after several failures a diet was found which will support growth in young guinea pigs, 200 to 275 gm. in weight and 3 to 5 weeks old. The diet contained extracted casein 30, sucrose or cornstarch 45.5, agar 5.0, salts 2.5, Crisco 2.0, and acetone extracted wheat germ 15.0 parts, with daily supplements of wheat germ oil 2 drops, ascorbic acid 8 mg., orange juice 3 cc., nicotinic acid 5 mg., and 0.25 cc. cod liver oil containing 0.04% carotene.

Four black guinea pigs of the same litter were placed on this diet in November, 1938, when their weights were 150 to 175 gm. All of them began to show dullness and metallic appearance of the fur in 6 to 8 weeks and one of them became completely grey by the last of January, 1939. The anti-grey factor was then given this animal but within 2 weeks it died still grey and in an emaciated condition. The fur of the other guinea pigs on the diet remained dull and metallic but did not grey and eventually all become cachectic and died. The guinea pig diet was not as deficient as that used for the rats because the wheat germ which it contained is by no means wholly free from the anti-grey factor.

Experiments with dogs and foxes

Eight young dogs were placed on a purified diet at 4 to 6 weeks of age with supplements of thiamin chloride, riboflavin and the wheat germ autolysate used for the rat experiments. Later crystalline synthetic vitamin B_6 [10] was used instead of the wheat germ preparation. Nicotinic acid was also given in some cases but not in all. The basal diet consisted of extracted casein 45.8, sucrose 20.9, cornstarch 19.4, Crisco 10.0, salts 2.4, and $CaCO_3$ 1.5 parts, and was supplemented by small amounts of carotene-reinforced cod liver oil.

Two black part-Boston bull female terriers were placed on this diet with nicotinic acid supplements, 3 mg. daily, late in January, 1939, at the age of 7 weeks when they weighed 1.7 kg. each. After 36 days their condition was serious, with

[10] We are indebted to Merck and Company for a supply of synthetic crystalline vitamin B_6.

frequent bloody diarrhea, stationary weight, and failure of appetite. Small doses of a filtrate concentrate were then given for 3 days with immediate improvement but after 30 days the symptoms recurred. The filtrate preparation was given for 4 days with immediate improvement again. After 40 days the weights again remained stationary.

After 2 to 4 months on the diet the intensely black fur of these dogs began to change to a reddish brown, first on the back of the neck then around the eyes and under the chin, and finally the fur was dull greyish over nearly the entire body surface. The teeth of both dogs rapidly discolored and pitted and the gums were somewhat reddish and swollen after 128 days on the diet. After 9 months on the deficient diet one of the dogs was given daily doses of the concentrated yeast filtrate preparation. After a few weeks repigmentation of the follicles and hair was clearly evident, the appetite had improved and the first estrus was established when the animal was 12 months of age. The other dog maintained on the deficient diet became steadily more emaciated, exhibited a bloody diarrhea and failed to show estrus, and was sacrificed in December, 1939, when moribund.

These and other dogs which received nicotinic acid but no filtrate preparation were affected more seriously by the deficiency than those which received neither of these factors.

Six young silver foxes at 2 months of age were placed on the diet used for the dogs, three receiving and three not receiving filtrate preparation, but all getting nicotinic acid and the other vitamin supplements. One fox on the deficiency diet died in 6 weeks and the other two grew a new coat of silvery white fur. Large mottled thymuses were found in the three animals on the deficient diet when sacrificed at 9 months of age, but no thymuses were evident in the three which received the filtrate preparation. A full account of this experiment will be published later. Survival of thymus tissue was detected also in some but not all of the rats which had greyed due to the deficiency.

The occurrence of unmistakable depigmentation in these young dogs and in one guinea pig as well as in several hundred rats and in two young silver foxes as a result of filtrate factor deficiency justifies the assumption that the greying phenomenon is truly a deficiency symptom. The concurrent skin and gland changes seen in the rats may well be suspected as being products of the same mechanism. These changes are typical of senescence, a process of obscure etiology. Perhaps the ageing process is conditioned by an increasing need for this sparsely distributed factor. In any case further study of the interrelation of the adrenal cortical and other hormones, the B vitamins and the phenomena of ageing appears justified.

SUMMARY

1. Young black or hooded rats were fed a basal diet adequate except with respect to the B vitamins and supplemented by thiamin chloride, riboflavin and a wheat germ preparation rich in vitamin B_6. The animals grew fairly well but within 6 to 10 weeks developed a greying of the fur, usually in bilateral patterns. Certain skin eruptions and in some cases persistent large skin ulcers appeared in many of the rats subsisting on this diet for several months.

2. Filtrates from fuller's earth-treated extracts of rice bran, yeast, liver, crude cane molasses and alfalfa in every case cured these symptoms but restored normal growth in varying degrees. Two filtrate factors appear to be involved, termed for convenience the anti-grey factor and the rat-growth filtrate factor.

3. Injection of commercial adrenal cortex and thyroid extracts cured the greying slowly but did not restore growth.

4. Histological study of adrenal glands, thyroids, skin and testes of these and similar rats deficient in vitamin B_6 or riboflavin showed that serious damage occurs in these tissues in the grey rats without corresponding changes in non-grey rats.

5. Lactation failed nearly completely in all rats placed on the filtrate factor-deficient diet on either the day of mating or the day of littering.

6. One guinea pig and eight young dogs have shown greying of the fur when subsisting on this deficient diet. The growth of the dogs was subnormal but the animals have survived for several months in spite of progressive greying of the hair, occasional diarrhea and poor appetite. Two young silver foxes were likewise affected.

7. The possible dependence of cortical adrenal and other gland function upon one of the members of the B complex and the relation of senescent changes to this deficiency are suggested.

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AVAILABILITY TO WHITE RATS OF PHOSPHORUS IN LESPEDEZA SERICEA AND ALFALFA HAYS

DOROTHY E. WILLIAMS, FLORENCE L. MacLEOD AND ELISE MORRELL

Department of Home Economics, Agricultural Experiment Station, University of Tennessee, Knoxville, in cooperation with the Tennessee Valley Authority

THE PROBLEM

According to Thomas ('38) variations in phosphorus of 250% may occur within the same species of plant grown on different soil types. Orr ('29) discussed the variation in mineral content of pasture plants. Weathers ('38) presented data showing differences due to soil type on mineral content in certain hay crops in Tennessee. Auchter ('39) ably summarizes the new trend in agricultural thinking which emphasizes the interrelation of such factors as soils, plants, and animals, and efforts directed toward improving the nutritional quality of crops as well as increasing their yields. The aim of the present investigation is to compare the availabilty to the animal of the phosphorus of a low phosphorus hay and of a high phosphorous hay.

EXPERIMENTAL PROCEDURE FOR LESPEDEZA SERICEA

Rapidly-growing male white rats, 30 to 31 days old, and weighing from 43 to 79 gm., were used for a preliminary study of availability of phosphorus in hays.

Lespedeza sericea [1] was the type of hay studied in the first set of experiments. Two samples of stems, one of low and

[1] The samples of lespedeza with analyses for minerals, nitrogen, and fiber were obtained from E. K. Weathers, Assistant General Chemist, U. T. Experiment Station.

one of high phosphorus content, and two samples of leaves of low and high phosphorus content were incorporated in four experimental diets. The rations as indicated in table 1 were so constructed that energy, fiber, mineral, and vitamin contents were identical for all animals in a given series, insofar as was possible, and all essential nutrients were present in adequate amounts with the exception of phosphorus. The amount of phosphorus for each diet was approximately 0.16% chosen on the basis of careful work done by Utley and MacLeod ('35) in this laboratory in constructing a diet which would supply either the minimal or slightly under the minimal amount of phosphorus for normal growth and phosphorus retention—an important consideration as emphasized by Mitchell and McClure ('37). In the control diet all of the phosphorus was supplied by salt mixture, whereas in the experimental diets 0.08% was supplied by salt mixture in each case and the remaining 0.08% by lespedeza. To make sure that animals could not be maintained in a normal nutritive condition on the 0.08% phosphorus supplied by the salt mixture without recourse to any phosphorus from lespedeza, four animals on the control diet containing 0.16% phosphorus as salt mixture were paired with four litter mates on a similar diet containing only 0.08% phosphorus as salt mixture. Food consumptions were kept uniform. To make the phosphorus content of all the experimental diets the same, different amounts of lespedeza were included for each diet. Calcium carbonate was added when necessary to supply 0.51% in each diet. This amount is adequate for normal growth and bone development. Thus the same Ca/P ratio of approximately 3 was maintained for all diets. To determine whether this Ca/P ratio was a disturbing factor in itself, animals on the control diet having a Ca/P ratio of 3 were paired with others on a control diet in which the calcium was reduced to give a Ca/P ratio of 1.5.

Comparisons were made by the paired feeding method between stems and leaves of low phosphorus content, 0.13% (0.29% P_2O_5) and 0.18% (0.41% P_2O_5) respectively, and between stems and leaves of high phosphorus content, 0.27%

(0.61% P_2O_5) and 0.26% (0.59% P_2O_5) respectively. A direct comparison was also made between the low phosphorus leaves and high phosphorus leaves. Since the phosphorus of feeds is often reported as phosphoric acid, the parenthetic values refer to the phosphorus in this form. Inclusion of control animals converted the paired to a triplicate feeding method. This method involved the use of three animals of the same sex, litter, and approximately the same weight, one animal being put on the control diet, and one each on the experimental diets being compared. The food consumption of the trio was kept the same by limiting two animals to the food intake of the one which ate the least. Thus, differences in growth and phosphorus retention due to varying food consumptions were ruled out. For the sake of comparison, some animals were allowed to eat each diet ad libitum.

The animals were started on the experimental diet at 30 to 31 days of age and killed at 60 to 61 days of age. The rats were ashed at approximately 550°C. and analyzed for phosphorus by the volumetric method essentially as approved by the Association of Official Agricultural Chemists ('35).

RESULTS FOR LESPEDEZA SERICEA

Table 2 summarizes the average results obtained for all animals. In both the direct and indirect comparison, rats on the diets containing low phosphorus lespedeza were less thrifty animals than their paired controls consuming the same amount of food whether judged on the basis of body weight, body length, or the amounts of phosphorus contained in the body. The animals on the diet containing the high phosphorus lespedeza much more nearly approached the control animals in regard to the various criteria used. Statistical analyses [2] (see table 2), using the phosphorus content of the body at 60 days of age as the criterion for comparison of the various diets, afford a mathematical interpretation of the data.

[2] Acknowledgment is made of the generous statistical aid received from Dr. B. L. Wade, Senior Geneticist, U. S. Regional Vegetable Breeding Laboratory, Charleston, S. C.

TABLE 1

Distribution of foodstuffs among the various constituents of the lespedeza and alfalfa diets[1]

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
DIETS	HAY IN DIET	PROTEIN FROM			CARBOHYDRATE FROM			FAT FROM BUTTER	FIBER FROM			CALCIUM FROM				PHOSPHORUS FROM			
		Hay	Lact.	Total	Hay	Starch[2]	Total		Hay	Agar	Total	Hay	Lact.	$CaCO_3$	Total	Hay	Lact.	H_3PO_4	Total
	%	%	%	%	*approx.* %	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Lespedeza Ca/P = 3																			
Control	0	0	18	18	0	40	40	8	0	28	28	0	0.036	0.476	0.512	0	0.025	0.125	0.150
Low P stems	63	5	13	18	28	10	38	8	28	0	28	0.435	0.026	0.050	0.511	0.080	0.018	0.067	0.165
Low P leaves	44	7	11	18	26	12	38	8	9	19	28	0.488	0.022	0	0.510	0.080	0.015	0.069	0.164
High P stems	30	3	15	18	13	26	39	8	13	15	28	0.198	0.030	0.280	0.508	0.080	0.021	0.062	0.163
High P leaves	31	6	12	18	17	21	38	8	6	22	28	0.369	0.024	0.120	0.513	0.080	0.016	0.068	0.164
Alfalfa Ca/P = 3																			
Control	0	0	18	18	0	59	59	8	0	9	9	0	0.036	0.449	0.485	0	0.027	0.128	0.155
Low P	37	7	11	18	19	38	57	8	9	0	9	0.477	0.022	0	0.499	0.079	0.017	0.070	0.166
High P	23	6	12	18	10	46	56	8	4	5	9	0.288	0.024	0.223	0.535	0.080	0.018	0.065	0.163
Alfalfa Ca/P = 5																			
Control	0	0	18	18	0	55	55	8	0	13	13	0	0.036	0.653	0.689	0	0.027	0.118	0.145
Low P	56	10	8	18	29	22	51	8	13	0	13	0.717	0.016	0	0.733	0.120	0.012	0.030	0.162
High P	35	8	10	18	15	36	51	8	8	5	13	0.371	0.020	0.348	0.739	0.123	0.015	0.024	0.162

[1] Columns 1, 3, 6, 8, 10, plus 4% salt mixture and 2% cod liver oil give the percentage compositions for each diet.

[2] Yeast extract was incorporated with the starch.

With diets as nearly identical as possible in nutritive qualifications and with phosphorus designed to be the limiting factor for normal growth and bone development, it appears that a difference exists in the availability of phosphorus in the diet containing the lespedeza of low phosphorus content both as compared to the control and to that containing the lespedeza of high phosphorus content. That these differences in availability of phosphorus were due to lack of absorption and not to the type of phosphorus compound was indicated by the amounts of feces obtained from animals on the different diets (see table 2). In general the diets containing the higher percentages of low phosphorus lespedeza produced the largest amounts of feces, the diets containing smaller percentages of high phosphorus lespedeza produced the next largest amounts of feces, and the control diets with no lespedeza produced the least. The cause for these differences in weights of feces is not due to differences in bulk since the fiber contents of the diets were equalized, but may be related to the relative indigestibility of the nitrogen-free extracts of the diets containing lespedeza. In view of the fact of large amounts of feces and of definitely restricted growth and phosphorus retention of animals on the diet containing lespedeza of low phosphorus content, it seems possible that the large amount of hay necessary to supply 0.08% phosphorus was not digested sufficiently to make available for absorption the phosphorus contained within the cellulose plant walls. Since the animals on the diets containing lespedeza of high phosphorus content showed increased growth and phosphorus retention, indicating that the phosphorus of the lespedeza had been better utilized, it appears that the amounts of this high phosphorus lespedeza necessary to supply the 0.08% phosphorus were small enough to be well digested and assimilated.

Table 2 also compares the average amounts of phosphorus from each diet stored by the rats during the experimental period. Values for the amount of phosphorus in the food consumed and the amount of phosphorus in the rat at the end of the experiment were known by analysis. Using Sherman and

TABLE 2

Summary of average results for experimental animals in growth and phosphorus intake and retention including summaries from variance analyses for phosphorus content of rat at 60–61 days of age

EXPERIMENTAL GROUP	NO. OF CASES	WEIGHT			LENGTH OF TRUNK	TOTAL FECES DRY WEIGHT	FOOD CONSUMPTION WITH A.D.[1]	TOTAL P IN FOOD WITH A.D.	P IN RAT AT 60–61 DAYS WITH A.D.	P IN RAT AT 30–31 DAYS WITH A.D.	P STORED FROM FOOD WITH A.D.	PER CENT P FROM FOOD
		Initial net	Final net	Gain net								
		gm.	*gm.*	*gm.*	*cm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	%
Triplicate feeding												
Low P lespedeza												
Control	5	53	136	83	16.8	111	293±18	0.440±0.027	0.728 ±0.040	0.281±0.053	0.448±0.020	102
Stems	5	54	92	38	14.8	199	294±16	0.485±0.027	0.528[2]±0.032	0.284±0.038	0.242±0.016	50
Leaves	5	53	90	37	15.0	201	295±16	0.483±0.026	0.554 ±0.020	0.281±0.036	0.273±0.028	57
F value for diets									184[3]			
Significant difference									0.028			
High P lespedeza												
Control	5	46	124	78	16.2	99	323±7	0.484±0.010	0.652±0.048	0.244±0.028	0.408±0.020	84
Stems	5	47	113	66	15.4	140	324±9	0.529±0.014	0.651±0.038	0.249±0.040	0.402±0.018	76
Leaves	5	46	104	57	14.9	161	324±9	0.531±0.015	0.590±0.041	0.245±0.037	0.345±0.030	65
F value for diets									257[3]			
Significant difference									0.023			
Significant difference for low vs. high P lespedeza									0.026			
Lespedeza leaves												
Control	4	56	115	58	15.1	85	256±48	0.384±0.072	0.510±0.107	0.296±0.009	0.214±0.075	77
Low P	4	54	74	20	13.4	151	259±46	0.424±0.076	0.464±0.080	0.286±0.016	0.178±0.064	42
High P	4	54	91	37	14.0	135	259±45	0.425±0.074	0.523±0.075	0.284±0.009	0.239±0.067	56
F value for diets									3.35[4]			
Significant difference									0.048			
Ad libitum feeding												
Control	2	68	181	113	18.0	150	455±19	0.683±0.028	0.932±0.031	0.361±0.022	0.572±0.010	84
Low P stems	1	72	132	60	15.8	234	405	0.668	0.738	0.382	0.356	53
Low P leaves	4	63	122	60	15.6	267	454±31	0.745±0.051	0.705±0.060	0.332±0.031	0.374±0.031	50
High P stems	1	56	166	110	17.3	193	454	0.740	0.920	0.297	0.623	84
High P leaves	5	67	161	94	17.0	247	492±18	0.803±0.030	0.803±0.039	0.357±0.014	0.446±0.026	68
Paired feeding												
Control Ca/P = 3	4	60	158	98	17.2	115[5]	349±16	0.524±0.024	0.793±0.039	0.318±0.029	0.475±0.062	91
Control Ca/P = 1.5	4	60	169	109	17.5	101[5]	349±17	0.559±0.026	0.886±0.013	0.317±0.028	0.569±0.016	102

High P stems	1	56	166	110	17.3	193	454	0.740	0.920	0.297	0.623	84
High P leaves	5	57	161	94	17.9	247	499±18	0.803±0.030	0.903±0.039	0.367±0.014	0.546±0.026	68
Paired feeding												
Control Ca/P = 3	4	60	158	98	17.2	115[5]	349±16	0.524±0.024	0.793±0.039	0.318±0.029	0.475±0.062	91
Control Ca/P = 1.5	4	60	169	109	17.5	101[5]	349±17	0.559±0.026	0.886±0.013	0.317±0.028	0.569±0.016	102
F value for diets									9.85[4]			
Significant difference									0.093			
Control P = 0.16%	4	54	118	64	15.7	90	278±13	0.417±0.020	0.624±0.034	0.286±0.005	0.337±0.039	81
Control P = 0.08%	4	54	105	51	15.8	86	276±12	0.210±0.010	0.502±0.008	0.288±0.009	0.215±0.015	102
F value for diets									27.09[6]			
Significant difference									0.087			
Triplicate feeding												
Alfalfa Ca/P = 3												
Control	5	57	157	100	16.7	35	303±60	0.470±0.092	0.755±0.150	0.304±0.046	0.451±0.103	96
Low P	5	57	143	86	16.1	74	304±59	0.504±0.098	0.725±0.125	0.301±0.046	0.423±0.080	83
High P	5	57	145	88	16.2	62	303±60	0.493±0.099	0.752±0.130	0.302±0.043	0.450±0.087	91
F value for diets									8.15[3]			
Significant difference									0.037			
Alfalfa Ca/P = 5												
Control	5	54	110	56	15.9	34	222±40	0.322±0.058	0.584±0.055	0.288±0.015	0.296±0.056	92
Low P	5	54	88	34	14.7	78	223±39	0.361±0.063	0.523±0.054	0.286±0.011	0.237±0.054	66
High P	5	54	100	46	15.5	57	224±39	0.363±0.062	0.579±0.057	0.287±0.010	0.291±0.062	80
F value for diets									8.15[3]			
Significant difference									0.037			
Significant difference for Ca/P = 3 vs. Ca/P = 5									0.021			

[1] A.D. = average deviation.
[2] Average of four animals.
[3] F value exceeds the 1% point.
[4] F value does not exceed the 5% point.
[5] Average of two animals.
[6] F value exceeds the 5% point.

Quinn's ('26) average value of 0.53% phosphorus in the body of rats at 28 days of age, the phosphorus present in the body at the beginning of the experiment was estimated. From these data the amount of phosphorus retained from the food during the experimental period was calculated. In the series of diets containing stems and leaves of lespedeza low in phosphorus, the control animals retained all the phosphorus of the diet whereas the experimental animals retained only about one-half the amount supplied by the food. Considering that in the experimental diets one-half of the phosphorus was supplied by salt mixture and the other half by lespedeza, it seems probable that here the rats had been able to use the phophorus from the readily assimilable salt mixture but that most of the phosphorus in the lespedeza was unavailable. In the series of diets containing stems and leaves of lespedeza high in phosphorus, the estimations indicated that the control animals retained, on the average, 84% of the phosphorus of the diet. In the case of the experimental animals, we also find that both those on stems and on leaves stored considerably more than one-half the phosphorus of the food. In other words, in both cases a considerable part of the phosphorus of the high phosphorus lespedeza was available to the animal. Similar relationships hold when the low and high phosphorus lespedeza leaves were compared directly.

When animals on the diet containing lespedeza low in phosphorus content were allowed to eat ad libitum, their growth and phosphorus retentions were improved over those of animals whose diets had been restricted by the triplicate feeding method, but they did not equal those of animals allowed to eat the control diet ad libitum. When animals on the diet of lespedeza high in phosphorus content were allowed to eat ad libitum, their growth and phosphorus retentions were not only improved over those whose diets had been restricted but they approached the ad libitum control animals in growth and equaled them in phosphorus retention.

The results of the experiment to test the disadvantage resulting from the disturbance in the Ca/P relationship, as

shown in table 2, gave slightly superior results in favor of the 1.5 to 1.0 ratio. Rates of growth, 158 and 169 gm., and phosphorus content of the body at 60 days, 0.793 and 0.886 gm., for Ca/P ratio of 3 and 1.5 to 1.0, respectively, were within normal limits as compared with those obtained by Sherman and Quinn ('26) for male rats at 61 days, 142 gm. and 0.814 gm. respectively. A variance analysis of these data indicates no difference of statistical significance between the two diets. Therefore, there could have been no serious disturbance in the present experiments as a result of an adverse Ca/P ratio.

At the termination of the experiment designed to show that 0.08% phosphorus is not sufficient for normal growth and phosphorus retention, the rats on the diet with 0.08% phosphorus contained, on the average, 0.502 gm. phosphorus in contrast to 0.624 gm. phosphorus for their paired litter mates consuming the same amount of food on the 0.16% level of phosphorus. Animals allowed to eat the diet containing 0.16% phosphorus ad libitum had, on the average, 0.932 gm. body phosphorus at the end of the experimental period. It is clear that 0.08% phosphorus in the diet is not enough to allow for normal growth and phosphorus retention.

The value of the paired feeding method is well illustrated in these experiments as a method of comparing two diets when the retention of a particular element, in this instance phosphorus, is the chief criterion for testing the diets rather than rate of growth. Table 3 indicates that, with no other change in the diet, both rate of growth and phosphorus content of the body increased with increased food consumption.

TABLE 3

Effect of food consumption on growth and phosphorus content of body

NUMBER OF CASES AVERAGED	FOOD CONSUMPTION	GAIN IN WEIGHT OVER 30-DAY EXPERIMENTAL PERIOD	P CONTENT AT END OF 30-DAY EXPERIMENTAL PERIOD
	gm.	*gm.*	*gm.*
2	208±12	46± 5	0.404±0.002
5	270± 5	74±12	0.648±0.056
15	323±17	91±10	0.704±0.069
2	455±19	123± 3	0.932±0.031

EXPERIMENTAL PROCEDURE FOR ALFALFA

It seemed desirable to test similarly samples of another type of leguminous hay varying in phosphorus content. Alfalfa was chosen because of the high place assigned to it as a feed. A preliminary difficulty arose in obtaining samples of alfalfa having as low a phosphorus content as that of the low phosphorus lespedeza. Out of five samples of alfalfa, two contained the high phosphorus content of 0.35% (0.80% P_2O_5) and one the relatively low phosphorus content of 0.21% (0.48% P_2O_5). The difference between the highest and lowest phosphorus contents is 0.14%, which is just about the difference between the high and low phosphorus contents of lespedeza used in the preceding experiments. The low phosphorus alfalfa, however, is almost as high as the high phosphorus lespedeza. Two sets of experiments were therefore initiated. In the first, the diets were constructed similarly to the lespedeza diets in that 0.08% phosphorus was supplied from alfalfa and 0.08% from a salt mixture. This meant that much smaller percentages of alfalfa were needed to supply the phosphorus than were required of the lespedeza. In the second set, 0.12% phosphorus was supplied by the alfalfa and only 0.04% by a salt mixture. This required amounts of alfalfa very similar to the amounts of lespedeza used. Since alfalfa is rich in calcium, the increased percentage of alfalfa in the diet increased the Ca/P ratio of 3 in the previous diets to one of 5. For the sake of brevity, the alfalfa diets deriving 0.08% of their phosphorus from alfalfa and 0.08% from salt mixture will be referred to as Ca/P = 3 diets, and those deriving 0.12% phosphorus from alfalfa and 0.04% from salt mixture will be referred to as Ca/P = 5 diets. The Ca/P ratio was kept the same for the three diets being compared in each series. No separation was made between stems and leaves, the comparisons being limited to that between an alfalfa high in phosphorus and one relatively low in phosphorus. The rest of the experimental procedure was similar to that followed with the lespedeza.

RESULTS FOR ALFALFA

For the series of Ca/P = 3 diets, reference to table 2 shows slight differences between the animals on the low phosphorus hay and either the controls or animals on the high phosphorus hay in respect to the various criteria used. These differences are too small, however, to be definitely considered significant although they approach significance. The difference between the animals on the low phosphorus hay and the controls is 0.030, whereas 0.037 is required for significance.

As in the lespedeza experiments, it is noted for the Ca/P = 5 diets that a high percentage of phosphorus from the food, 92% and 80%, was utilized by the controls and animals on the high phosphorus alfalfa, respectively, whereas only 66% was utilized by those on the low phosphorus alfalfa. This again correlates with the large amount of feces eliminated by the low phosphorus group and the smaller amounts for the high phosphorus and control groups.

In other words, it has been shown for alfalfa as well as for lespedeza that the phosphorus of a hay of low phosphorus content, necessitating the feeding of a large amount of hay to meet the phosphorus requirement for normal growth and bone development, is not so available to growing white rats as the phosphorus of the same variety of hay having a higher phosphorus content and therefore requiring a smaller amount to supply the phosphorus needs.

SUMMARY OF RESULTS

1. When the experimental diets contain phosphorus at a minimal level of adequacy and all other nutrients at optimal levels and the same for all diets, the phosphorus of a low phosphorus lespedeza sericea or alfalfa hay is less available to the rat for growth and bone development than the phosphorus of a high phosphorus hay of the same type.

2. A diet containing 0.16% phosphorus and a Ca/P ratio of 3 to 1 is just about minimal for normal growth and phosphorus retention when the diet is fed ad libitum or in slightly restricted amounts.

3. The paired feeding method eliminates the variable of different food consumptions.

CONCLUSION

On the basis of the above results, it would appear that rats consuming a relatively large percentage of hay in order to supply even the minimal amount of phosphorus, lose some of the nutrients, including phosphorus, through a lack of absorption due to increased elimination. With the phosphorus at the minimal level, it constitutes the limiting factor for growth and any appreciable loss of phosphorus results in symptoms of phosphorus deficiency. A hay having a higher phosphorus content and furnishing the minimal amount of phosphorus with a smaller amount of hay can apparently be retained in the intestines long enough for sufficient digestion to make the phosphorus available to the white rat.

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OBSERVATIONS ON THE AMOUNT OF ASCORBIC ACID REQUIRED TO MAINTAIN TISSUE SATURATION IN NORMAL ADULTS [1,2]

E. NEIGE TODHUNTER AND RUTH C. ROBBINS
College of Home Economics, State College of Washington, Pullman

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Belser, Hauck and Storvick ('39) have reported a method for determining the minimum intake of ascorbic acid which will just maintain the tissues in a state of complete saturation as judged by the urinary excretion of ascorbic acid in response to various test doses.

In September, 1938, Dr. Hazel Hauck kindly sent us the details of the procedure being used in her investigation. This method has been used in our laboratory for three subjects and is reported here because it confirms the findings of Belser, Hauck and Storvick ('39) and also because data on blood plasma ascorbic acid were obtained which give additional information on the problem of tissue saturation and the possible requirement of vitamin C.

EXPERIMENTAL

Three college women who served as subjects lived on the basal diet and followed the procedure exactly as described by Belser, Hauck and Storvick ('39).

The titration method of Bessey and King ('33), modified to use 3% metaphosphoric acid as the extracting reagent, was used for the vitamin C determination of all the foods of the

[1] Published as scientific paper no. 425, Agricultural Experiment Station, State College of Washington.

[2] This investigation is part of the regional project of the Northwest States on the ascorbic acid metabolism of college students.

basal diet except the beets and prunes. For these pigmented foods, the metaphosphoric acid extract was titrated by the method of McHenry and Graham ('35). Assuming that ascorbic acid was the only substance contributing to the reducing values as determined by titration, the basal diet, in the weighed amounts consumed daily, contained 20 mg. of ascorbic acid obtained as follows: pears and juice 2.8 mg., beets and juice 11.1 mg., evaporated milk 0.7 mg., carrots and juice 1.6 mg., prunes 2.8 mg., beef 1.0 mg. This basal diet contained approximately twice as much ascorbic acid as that reported by Belser and co-workers ('39); the beets accounted for the difference. Ascorbic acid supplements [3] were taken at breakfast time.

The experimental period lasted through March, April, and May, 1939. Twenty-four-hour samples of urine were collected in jars containing a preservative of sulphuric acid, hydroxyquinoline and toluene as recommended by Sendroy ('37). Urine preserved in this manner for 24 hours showed a loss of ascorbic acid of less than 5%.

The subjects were of the following height and weight, A.F.: 51.7 kg., 161.0 cm.; W.B.,: 51.3 kg., 162.0 cm.; N.T.: 66.7 kg., 173.5 cm.

Blood samples were taken in the morning before breakfast. About 0.8 cc. of blood was collected in a small vial from a finger prick and was analyzed immediately for ascorbic acid using the micro method of Farmer and Abt ('36).

RESULTS AND DISCUSSION

Urine. The response in urinary excretion to a 400 mg. test dose of ascorbic acid following a 4-day period when 200 mg. daily was taken, was determined for three successive periods for each subject. The daily excretion values are shown in table 1; not only were there differences in ascorbic acid excretion of individuals living on the same basal diet and with the same ascorbic acid intake, but there was also a definite

[3] Acknowledgment is made to Merck and Company for a generous supply of Cebione.

difference in response to the test dose by the same individual for the three periods. Subject A.F. also showed considerable variation in the day-to-day excretion. Both subjects A.F. and W.B. were found to have normal kidney function when tested for rate of phenolsulphonphthalein excretion.

TABLE 1

Blood plasma and urinary response to 400 mg. test dose of ascorbic acid following 200 mg. intake daily for 4 days by three human subjects

DAILY INTAKE OF ASCORBIC ACID IN ADDITION TO BASAL DIET		ASCORBIC ACID CONTENT					
		Urine: mg. per 24 hours			Blood:[1] mg. per 100 ml. plasma		
		A.F.	W.B.	N.T.	A.F.	W.B.	N.T.
Day	*mg.*						
First	200	139	...	210			
Second	200	133	167	177			
Third	200	105	167	187			
Fourth	200	131	190	185			
Fifth	400	170	281	277	1.70	1.84	1.48
Sixth	...				1.70	1.78	1.69
First	200	156	153	176			
Second	200	140	189	141			
Third	200	125	179	136			
Fourth	200	98	183	161			
Fifth	400	285	272	327	1.63	1.34	1.31
Sixth	...				1.55	1.29	1.42
First	200	135	184	193			
Second	200	121	191	156			
Third	200	148	168	166			
Fourth	200	111	...	163			
Fifth	400	270	229	308	1.52	1.30	1.42
Sixth	...				1.33	1.32	1.40

[1] Blood plasma determinations were made on the morning of taking 400 mg. ascorbic acid and on the following morning.

The lowest level of ascorbic acid which was excreted in response to the 400 mg. test dose was taken as the criterion for complete saturation for each subject and was as follows: subject A.F., 170 mg.; subject W.B., 229 mg.; subject N.T., 277 mg.

Time did not permit investigation of as many levels of intake as Hauck and co-workers used; nevertheless, sufficient

data were obtained to indicate the probable requirement of each subject in order to maintain tissue saturation under the specified conditions. These data are summarized in table 2. Subject A.F. showed tissue saturation as judged by the 400 mg. response following an intake of 70 mg. daily for 6 days. Daily excretions of this subject were variable and a lower response was obtained after 100 mg. daily intake than when receiving 90 mg. daily. A similar variation in response has been noted by Belser, Hauck and Storvick ('39) with some of their subjects. Subject W.B. attained tissue saturation following a daily intake of 60 mg.; at the 70 mg. level of intake there was practically no increase in the 400 mg. response and when the test at this level was repeated the 400 mg. response was lowered. This further confirmed our previous observation that there is a wide range in excretion of ascorbic acid for the same individual under the same conditions of ascorbic acid intake. Subject N.T. gave approximately the required response to the 400 mg. test dose following a 6-day intake of 90 mg. of ascorbic acid daily.

From the data in table 2, it was concluded that, in addition to the basal diet, subject A.F. required approximately 70 mg., subject W.B. 60 mg., and subject N.T. more than 90 mg. to maintain tissue saturation.

When the ascorbic acid intake of 20 mg. daily from the basal diet was added to these values and the requirement then calculated on the basis of body weight the following were the requirements per kilogram of body weight: A.F., 1.7 mg., W.B., 1.6 mg. and N.T., more than 1.6 mg. Belser and co-workers ('39) reported that from 1.0 to 1.6 mg. per kilogram was required by their subjects.

Blood. It is possible that the blood plasma levels of ascorbic acid are a more reliable index of the state of ascorbic acid nutrition than the urine levels. There are many sources of error in the urinary determination and little is known of the amount or rate of destruction of ascorbic acid in its passage through the kidneys and ureters and while retained in the bladder.

TABLE 2

Blood plasma and urinary response to 400 mg. test dose following different levels of ascorbic acid intake [1] for 6-day periods

SUBJECT A.F.			SUBJECT W.B.			SUBJECT N.T.		
Ascorbic acid			Ascorbic acid			Ascorbic acid		
Intake	Urine	Plasma	Intake	Urine	Plasma	Intake	Urine	Plasma
mg.	*mg. per 24 hr.*	*mg. per 100 ml.*	*mg.*	*mg. per 24 hr.*	*mg. per 100 ml.*	*mg.*	*mg. per 24 hr.*	*mg. per 100 ml.*
40 [2]	21	1.68	60	90	1.48	40	27	1.47
40 [2]	22	....	60	79	1.41	40	16	....
40 [2]	18	....	60	70	1.34	40	22	....
40 [2]	5	....	60	39	1.14	40	21	1.16
40 [2]	15	....	60	32	1.16	40	14	1.28
40 [2]	8	....	60	33	....	40	19	1.08
400	153	1.17	400	242	1.12	400	130	1.10
		1.67 [3]			1.61 [3]			1.64 [3]
70	62	1.50	70	70	1.42	80	114	1.50
70	29	1.36	70	63	1.29	80	72	....
70	12	1.28	70	58	1.18	80	85	1.37
70	26	1.04	70	64	1.04	80	68	1.29
70	33	....	70	57	....	80	54	1.14
70	16	1.06	70	47	1.12	80	50	1.16
400	175	1.06	400	250	1.12	400	245	1.19
		1.48 [3]			1.66 [3]			1.60 [3]
90	44	1.36	70	95	1.52	90	110	1.41
90	46	....	70	76	....	90	82	1.23
90	24	1.34	70	71	1.28	90	83	....
90	40	1.26	70	50	1.26	90	83	1.11
90	38	1.19	70	46	1.14	90	71	1.03
90	68	1.21	70	43	1.10	90	69	....
400	280	1.12	400	210	1.14	400	263	0.99
		1.41 [3]			1.58 [3]			1.56 [3]
100	120	1.60						
100	25	....						
100	102	1.33						
100	53	1.31						
100	28	1.26						
100	31	1.06						
400	248	1.04						
		1.43 [3]						

[1] The intake noted was taken as crystalline ascorbic acid in addition to the basal diet.

[2] Ascorbic acid was taken as red raspberries calculated to contain 40 mg. ascorbic acid.

[3] Blood plasma values 24 hours after ingestion of 400 mg. dose.

In the first part of the study, blood determinations were made in the morning when the 400 mg. test dose was given, and on the following morning; i.e., at the end of the 24-hour urinary collection period. The data are summarized in table 1; for each subject there was practically no difference in blood level before and after taking the 400 mg. test dose. It was concluded that the subjects were saturated before taking the test dose since the 400 mg. test dose did not raise the blood level of ascorbic acid; during the 24-hour excretion period the excess ascorbic acid was removed from the blood, either being oxidized to some other form or excreted in the urine. The plasma levels for these saturated subjects for the three periods ranged from 1.33 to 1.70 for A.F., from 1.29 to 1.84 for W.B., and from 1.31 to 1.69 for N.T. These values are in agreement with the reports of Faulkner and Taylor ('38) and others (Wright, '38; Lund, '37; Goldsmith and Ellinger, '39) that the renal threshold for ascorbic acid is approximately 1.4 mg. per 100 ml. of blood plasma.

In the second phase of the investigation, daily determinations of the blood plasma ascorbic acid were made for all subjects, except for subject A.F. when on the 40 mg. intake level. The individual daily blood values are given in table 2 and represent the plasma content of ascorbic acid on the morning of each day of ascorbic acid intake and also on the morning following the 400 mg. test dose. The blood values at the beginning of each period ranged from 1.36 to 1.68 mg. for all subjects and indicated saturation; the blood level then fell during the 6-day period for each subject but was immediately raised to 1.4 mg. or above on the morning following the 400 mg. test dose.

Faulkner and Taylor ('38) reported that a maintained value of blood ascorbic acid at or above the threshold value corresponds to saturation, and below the threshold level corresponds to unsaturation. However, it is still an open question whether the blood level should be maintained at 1.4 mg. for optimum health; more than 120 mg. daily was required by each

subject to maintain this level. Wright ('38) has stated that the normal blood plasma level of ascorbic acid in adults lies between 0.7 and 1.3 mg. per 100 ml. The data in table 2 show that both subjects A.F. and N.T. had a blood level above 0.7 mg. when the total daily intake was 60 mg. (basal diet plus 40 mg.) of ascorbic acid, the values being 1.17 mg. and 1.10 mg. respectively.

If a blood plasma level of 0.7 mg. and an increase to 1.4 mg., 24 hours after taking a 400 mg. test dose is accepted as a measure of tissue saturation, then 60 mg. of ascorbic acid daily was above the requirement of these subjects. If a blood value maintained at approximately 1.4 mg. is taken as saturation then 120 mg. daily was inadequate.

SUMMARY

Using a method for estimating the minimum intake of ascorbic acid required to maintain the tissues in a state of complete saturation as judged by urinary excretion of ascorbic acid, the requirement for three adult subjects was found to be from 1.6 to 1.7 mg. per kilogram per day. The urinary excretion was found to vary considerably for the same individual even under carefully controlled conditions of ascorbic acid intake.

Values above 1 mg. of ascorbic acid per 100 ml. of blood plasma were obtained when the total daily intake was 60 mg.; more than 120 mg. daily were required to raise the blood plasma to 1.4 mg. The significance of the blood plasma levels in relation to saturation is discussed.

Grateful acknowledgment is made to Wilma Brewer and Mrs. Alva Fatzer for their loyal cooperation and assistance throughout the entire study.

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THE INDEPENDENCE OF THE ENDOGENOUS AND THE EXOGENOUS METABOLISM OF NITROGEN [1]

E. WISE BURROUGHS, HELEN S. BURROUGHS AND H. H. MITCHELL
Division of Animal Nutrition, University of Illinois, Urbana

(Received for publication December 7, 1939)

The theory of protein metabolism proposed by Folin ('05) distinguishes between two kinds of protein catabolism:

One kind is extremely variable in quantity, the other tends to remain constant. The one kind yields chiefly urea and inorganic sulphates, no kreatinin, and probably no neutral sulphur. The other, the constant katabolism, is largely represented by kreatinin and neutral sulphur, and to a less extent by uric acid and ethereal sulphates. The more the total katabolism is reduced, the more prominent become these representatives of the constant katabolism, the less prominent become the two chief representatives of the variable katabolism.

The constant type of metabolism Folin called the endogenous metabolism and because of its constancy and its independence of dietary protein, he referred it to the tissues of the body and assumed that it represented "an essential part of the activity which distinguishes living cells from dead ones." The variable protein metabolism was called the exogenous metabolism and was referred to the disposition of dietary protein.

Although the theory of Folin has dominated later investigations of this subject and has been confirmed in many of its details, no one of its basic theses has escaped at least an occasional denial, because of evidence that seems impossible to reconcile with it.

[1] The substance of this paper was taken from a thesis submitted by E. Wise Burroughs to the Graduate School of the University of Illinois in partial fulfillment of the requirements of the degree of Doctor of Philosophy in Animal Husbandry, July, 1939.

The independence of the endogenous and exogenous types of metabolism has been denied on the basis of indications that certain amino acids incorporated in the diet may either stimulate or depress the endogenous metabolism. Such indications, to be valid, should be obtained with animals that have been so prepared that the urinary nitrogen is all of endogenous origin, including no nitrogen of immediate dietary origin nor any originating from the catabolism of the labile stores of protein in the body, sometimes referred to as "deposit protein." They should also be sufficiently definite and reproducible so that they can be clearly distinguished from the normal variations in the output of endogenous nitrogen.

The most serious challenge to the independence of the endogenous and exogenous types of protein (nitrogen) metabolism is provided by the recent investigations of Schoenheimer and associates, using isotopic nitrogen to label the amino acids whose metabolism was studied. These investigations have revealed a rapid and extensive interchange of nitrogen between dietary amino acids and tissue proteins that presumably results from chemical reactions involving the opening of peptide linkages, deamination, reamination or even transamination of amino acid residues, with re-entrance into positions in the tissue protein molecules left vacant by the rupture of peptide linkages. In their most recent publication, Schoenheimer and his colleagues (Schoenheimer, Ratner and Rittenberg, '39) conclude: "It is scarcely possible to reconcile our findings with any theory which requires a distinction between these two types of nitrogen," referring to endogenous and exogenous nitrogen.

However, it may reasonably be questioned whether these ingenious and revealing investigations of Schoenheimer bear any relation at all to the endogenous metabolism of Folin. Mature, or nearly mature, rats subsisting upon diets containing 16 to 18% of protein must have very considerable stores of proteins of the labile type ("deposit protein"), that are depleted and repleted readily with every fall and rise of the intake of dietary protein. Whipple ('38) speaks of the "fluid-

ity'' of these reserve stores, which he has shown in his own investigations to be readily available for the regeneration of hemoglobin and plasma protein after depletion by surgical hemorrhage and plasmapheresis, respectively. He distinguishes clearly, as Folin would, between these dispensable reserve proteins and the indispensable fixed proteins of the tissues. In all likelihood, the chemical reactions that Schoenheimer has detected by means of isotopic nitrogen between dietary amino acids and tissue proteins relate not to the fixed proteins of the cells, indispensable to their normal functioning, but to the dispensable reserve proteins, readily subject to mobilization by many experimental procedures and as readily reformed. In any studies of the endogenous metabolism, these protein stores must be reduced to zero by the continued feeding of diets containing only inconsiderable amounts of nitrogen.

The losses of nitrogen incurred in the endogenous catabolism presumably determine the minimum amounts of nitrogen required for the maintenance of life. The fact that these losses may be partially replaced by incomplete proteins, and simple amino acid mixtures, or even by individual amino acids, would seem to imply that the losses in the main do not represent the disintegration of tissue proteins, but of some nitrogenous constituents of the tissues of much simpler structure than proteins. On the other hand, Nielsen, Gerber and Corley ('39) and Nielsen and Corley ('39) have concluded that the diminished losses of body nitrogen brought about by supplementing a nitrogen-free diet with individual amino acids or simple amino acid mixtures, may be interpreted either as a retention (utilization) of part of their contained nitrogen, or as a sparing of an equivalent amount of nitrogen from the tissues. The latter interpretation involves a depression of the endogenous catabolism, distinctly at variance with Folin's theory.

The choice between these two interpretations seems a difficult one, although the fact that the creatinine excretions were stated to be constant is presumptive evidence that the endogenous nitrogen metabolism had not been depressed. Other evidence of the same sort that the endogenous metabolism is

neither depressed nor stimulated by dietary sources of nitrogen is afforded by the experiments of Terroine and Sorg-Matter ('27, '28) and of Smuts ('35) in which the endogenous metabolism is shown to be related closely to the basal metabolism of energy. Also, Mitchell, Burroughs and Beadles ('36) have concluded that the assumptions underlying the calculation of the biological values of proteins by the method developed in this laboratory, including the assumption of the independence of the endogenous and the exogenous metabolism of nitrogen, are essentially correct, since the values thus obtained are confirmed by an independent method.

The purpose of the investigations to be reported in this and two later papers was to study the endogenous metabolism of nitrogen by the use mainly of pure amino acids and mixtures of pure amino acids as the sole considerable source of dietary nitrogen. In this paper the experiments reported relate to the possibility of depressing the endogenous metabolism by the ingestion of small amounts of individual amino acids, of simple mixtures of amino acids, or of a protein mixture (egg) of high biological value. If the urinary nitrogen excreted on a nitrogen-free diet represents the catabolism of the amino acids of tissue origin left over after certain specific requirements for hormone-precursors have been met, according to an opinion not infrequently expressed, then the feeding of amino acids that may serve such purposes, in small amounts, would be expected to result in a sparing of tissue protein and consequently in a disproportionately large reduction in the excretion of nitrogen in the urine.

PLAN AND METHODS

Albino rats of both sexes and of mature or approximately mature weight were used as subjects of the experiment. The plan of the experiment involved three consecutive experimental periods of 2 to 5 days each (generally 3 or 5) following a preliminary period of 5 or 6 days' duration. Throughout this time the rat received a nitrogen-free diet, supplemented

daily by 20 mg. of yeast concentrate [2] to provide the vitamins of the old B complex. During the second experimental period the rats received daily, separate from the basal ration, a small supplement of a single amino acid, a simple mixture of amino acids, or egg protein. The effect of the nitrogen supplement on the endogenous catabolism was assessed by comparing the excretion of urinary nitrogen of the second period with the average excretion of the first and third periods.

The nitrogen-free diet contained 63% starch, 10% sucrose, 12% lard, 8% butterfat, 5% salt mixture (modified from Osborne and Mendel to include cobalt, copper, and zinc), and 2% cod liver oil. An attempt was made to maintain constant the intake of food for each rat throughout its three experimental periods.

The rats were kept in individual metabolism cages and the collections of urine and feces were made according to the procedure used in this laboratory in the determination of the biological value of proteins. For a description of cages and procedure the article by Smuts ('35) may be consulted.

The feces of the different experimental periods were separated sharply by the use of markers, ferric oxide and chromic oxide being used alternately. The urine was completely removed from the urinary bladder daily, either by catheterization in the case of female rats, or by pressure applied over the ureters. With training, the animals could be induced to respond to the latter method as satisfactorily (as tested by the catheter) as to the former, and with much less time and trouble on the part of the operator.

The urine was preserved with sulfuric acid and toluene. Both feces and urine were analyzed for total nitrogen by the Kjeldahl-Gunning-Arnold method, and the urine was analyzed colorimetrically for creatinine, using a photo-electric colorimeter and a purified creatinine standard.

The validity of the method of detecting a possible effect of the nitrogenous supplements upon the endogenous metabolism

[2] From the Harris Laboratories, Tuckahoe, N. Y.

by comparing the urinary nitrogen of the second experimental period with the average nitrogen excretion of the first and third periods of nitrogen-free feeding, depends either upon a constancy of the output of endogenous nitrogen or upon a rectilinear change from period 1 to period 3. Preliminary tests upon three adult rats indicated that either condition may exist after a preliminary feeding period of 5 days.

TABLE 1

Average daily excretion of urinary nitrogen in the first and third periods of nitrogen-free feeding compared with the second period

RAT NO.	BODY WEIGHT	LENGTH OF EACH PERIOD	FIRST PERIOD	THIRD PERIOD	AVERAGE FIRST AND THIRD	SECOND PERIOD	DIFFERENCE
	gm.	*days*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
1	261	3	58	41	50	48	—2
2	236	3	55	45	50	51	1
3	236	3	57	42	50	45	—5
1	258	3	48	36	42	41	—1
2	230	3	51	40	45	45	0
3	232	3	45	36	40	42	2
1	255	3	41	33	37	36	—1
2	224	3	45	37	41	40	—1
3	227	3	42	35	38	36	—2
6	349	5	98	62	80	80	0
10	406	5	73	59	66	67	1
16	285	5	66	50	58	59	1
6	356	3	103	80	92	89	—3
10	410	3	76	66	71	69	—2
16	291	3	68	59	63	63	0
6	347	3	89	66	78	80	2
10	405	3	69	60	65	66	1
16	284	3	63	50	57	59	2
Average			64	50	57	57	0

To put the question to a quantitative test, six rats were fed the nitrogen-free diet for a period of 20 days. After the first 5 days, which were considered a preliminary adjustment period, urine collections were made for successive 3- or 5-day periods. Then for each rat, the average daily excretions of urinary nitrogen for series of three successive periods were studied by comparing the excretion of the middle period with the average excretion of the initial and final periods. The results of this study are summarized in table 1.

The results indicate that during the second period of specific nitrogen inanition the excretion of urinary nitrogen was approximately midway between that of the first and third periods, the positive and negative deviations from mid-position averaging approximately zero. The standard deviation of the differences between the excretions of the second period and the average excretions of the first and third periods is 1.88 mg. of nitrogen. From the values of the normal probability integral (Davenport and Ekas, '36), it may be computed that the deviations from zero corresponding to probabilities of a fortuitous event of 0.01, 0.02, and 0.03 are 4.38, 3.85, and 3.53 mg. of urinary nitrogen. This may be taken to mean that if in a middle test period, in which an amino acid supplement is fed, the daily urinary excretion of nitrogen deviates from the average daily excretion of the first and third periods of nitrogen-free feeding by 4 mg. or more, the deviation may be considered to be statistically significant. This implies, of course, that the endogenous metabolism pursues a constant or a rectilinearly changing course throughout the three experimental periods.

DISCUSSION OF EXPERIMENTAL RESULTS

The essential results of the experiment are collected in tables 2, 3 and 4. In tables 2 and 3, a positive difference (last column of tables) in the excretion of urinary nitrogen between the test period and the initial and final periods of nitrogen-free feeding would, if statistically significant, testify to the incomplete utilization of the nitrogenous supplement tested. Although the results for the various supplements used are not entirely concordant, there is evidence of incomplete utilization, or complete failure of utilization, in the case of tryptophane, phenylalanine, leucine, and cystine.

A negative difference, representing a lower excretion of urinary nitrogen in the middle test period than in the initial and final periods of complete nitrogen inanition, would represent, if statistically significant, a depression of the endogenous metabolism. Among the forty-three experiments on thirteen individual amino acids reported in table 2, only thirteen gave

TABLE 2

The influence of small amounts of individual amino acids on the endogenous metabolism of nitrogen

AMINO ACID TESTED	RAT NO.	AVERAGE BODY WEIGHT	DAILY N INTAKE IN 2ND PERIOD	DAILY URINARY NITROGEN EXCRETION				
				Initial N-free period	Final N-free period	Average of N-free periods	Amino acid period	Difference
		gm.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Threonine	10	191	4.7	41.9	34.4	38.2	39.7	+1.5
Threonine	11	226	4.7	55.3	38.9	47.1	49.9	+2.8
Threonine	12	231	4.7	50.7	40.3	45.5	47.9	+2.4
Isoleucine	10	191	4.3	34.4	38.3	36.4	36.6	+0.2
Isoleucine	11	226	4.3	38.9	42.3	40.6	41.2	+0.6
Isoleucine	12	231	4.3	40.3	41.2	40.8	41.5	+0.7
Tryptophane	5	357	7.0	74.7	58.3	66.5	76.8	+10.3
Tryptophane	7	379	7.0	80.6	57.1	68.8	72.9	+4.1
Valine	27	321	4.8	85.3	59.8	72.6	72.7	+0.1
Valine	36	279	4.8	67.5	53.7	60.6	63.2	+2.6
Valine	37	263	4.8	53.9	39.8	46.8	50.3	+3.5
Methionine	15	330	5.6	77.1	39.4	58.2	40.4	−17.8
Methionine	27	345	4.0	83.1	57.6	70.4	63.3	−7.1
Methionine	28	323	4.8	83.6	52.6	68.1	63.5	−4.6
Methionine	4	221	4.7	46.5	33.3	39.9	37.1	−2.8
Methionine	5	217	4.7	42.1	25.7	33.9	41.7	+7.8
Methionine	6	218	4.7	36.0	32.3	34.2	35.1	+0.9
Lysine	7	216	3.8	50.3	38.0	44.2	46.1	+1.9
Lysine	8	195	3.8	47.0	39.4	43.2	43.7	+0.5
Lysine	9	216	3.8	43.3	37.4	40.3	42.3	+2.0
Histidine	30	354	5.5	67.8	46.8	57.3	56.0	−1.3
Histidine	31	351	5.5	76.9	59.8	68.4	67.2	−1.2
Histidine	32	374	5.5	90.8	61.9	76.4	72.6	−3.8
Phenylalanine	33	399	6.0	79.3	51.4	65.4	69.6	+4.2
Phenylalanine	34	353	6.0	79.7	59.9	69.8	65.6	−4.2
Phenylalanine	35	308	6.0	76.8	71.8	74.3	80.2	+5.9
Leucine	19	347	6.0	74.6	56.9	65.8	69.3	+3.5
Leucine	29	361	6.0	99.0	78.4	88.7	101.4	+12.7
Leucine	36	351	6.0	80.8	54.1	67.4	77.2	+9.8
Arginine	4	227	6.0	47.7	46.5	47.1	45.9	−1.2
Arginine	5	221	6.0	48.1	42.1	45.1	41.2	−3.9
Arginine	6	222	6.0	40.7	36.0	38.4	40.9	+2.5
Cystine	11	322	4.7	64.1	61.6	62.9	64.3	+1.4
Cystine	12	329	4.7	64.1	53.7	58.9	56.2	−2.7
Cystine	13	336	4.7	73.9	53.9	63.9	55.0	−8.9
Cystine	11	308	11.4	61.6	48.7	55.1	56.6	+1.5
Cystine	12	318	11.4	53.7	50.4	52.0	61.2	+9.2
Cystine	13	325	10.6	53.9	47.6	50.8	55.5	+4.7
Tyrosine	12	349	6.0	68.9	65.3	67.1	70.7	+3.6
Tyrosine	13	396	6.0	103.6	74.0	88.8	79.6	−9.2
Glutamic acid	7	216	5.7	38.0	36.5	37.2	39.1	+1.9
Glutamic acid	8	195	5.7	39.4	32.7	36.0	36.3	+0.3
Glutamic acid	9	215	5.7	37.4	35.1	36.2	40.8	+4.6

negative differences and of these differences, only six exceeded in magnitude the 4 mg. previously taken as the limiting value between statistical insignificance and statistical significance. These six cases relate to methionine (three cases), phenylalanine (one case), cystine (one case), and tyrosine (one case). They were all obtained in experiments in which the urinary nitrogen in the first nitrogen-free feeding period was unusually high, and greatly in excess of the nitrogen excretion

TABLE 3

The influence of small amounts of simple mixtures of amino acids and of egg proteins on the endogenous metabolism of nitrogen

AMINO ACID MIXTURE OR PROTEIN TESTED	RAT NO.	AVERAGE BODY WEIGHT	DAILY N INTAKE IN 2ND PERIOD	DAILY URINARY NITROGEN EXCRETION				
				Initial N-free period	Final N-free period	Average of N-free periods	Amino acid or protein period	Difference
		gm.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Mixture no. 1[1]	1	236	4.11	58.3	44.7	51.5	43.1	−8.4
Mixture no. 1	2	235	4.11	58.7	35.5	47.1	37.0	−10.1
Mixture no. 1	3	233	4.11	58.3	42.6	50.5	41.8	−8.7
Mixture no. 1	1	222	4.11	41.7	37.2	39.4	43.1	+3.7
Mixture no. 1	2	230	4.11	33.0	32.9	32.9	31.9	−1.0
Mixture no. 1	3	223	4.11	37.0	31.4	34.2	36.8	+2.6
Mixture no. 2[2]	4	227	4.76	54.5	35.7	45.1	43.1	−2.0
Mixture no. 2	5	216	4.76	61.7	43.3	52.5	45.3	−7.2
Mixture no. 2	6	217	4.76	54.2	36.5	45.4	42.7	−2.7
Mixture no. 2	4	213	4.76	35.0	35.0	35.0	40.4	+5.4
Mixture no. 2	5	203	4.76	44.4	43.5	44.0	43.6	−0.4
Mixture no. 2	6	206	4.76	33.7	34.1	33.9	41.1	+7.2
Mixture no. 3[3]	7	201	4.44	53.9	35.6	44.8	35.0	−9.8
Mixture no. 3	8	213	4.44	52.5	29.0	40.8	34.0	−6.8
Mixture no. 3	9	192	4.44	60.1	31.7	45.9	38.0	−7.9
Mixture no. 3	7	190	4.44	34.3	31.3	32.8	33.2	+0.4
Mixture no. 3	8	204	4.44	28.2	29.1	28.6	28.8	+0.2
Mixture no. 3	9	182	4.44	33.1	30.0	31.5	29.7	−1.8
Mixture no. 4[4]	72	168	8.0	29.4	26.5	28.0	30.6	+2.6
Mixture no. 4	75	177	8.0	25.6	24.2	24.9	26.4	+1.5
Egg proteins	39	218	22.4	36.4	32.5	34.4	33.9	−0.5
Egg proteins	40	220	22.4	38.6	32.7	35.6	36.2	+0.2

[1] This mixture contained equal quantities of nitrogen from methionine, histidine, arginine, isoleucine and lysine.

[2] This mixture contained equal quantities of nitrogen from valine, threonine, phenylalanine, tryptophane and leucine.

[3] This mixture is a combination of mixtures 1 and 2 in equal proportions.

[4] The nitrogen of this mixture was distributed as follows: threonine 15, isoleucine 15, valine 12, methionine 12, tyrosine 10, tryptophane 6, norleucine 30.

in the final period of nitrogen-free feeding. Furthermore, in none of the six cases suggesting a depression of the endogenous metabolism was the nitrogen excretion of the amino acid period less than that of the final period of nitrogen-free feeding. These facts indicate that the decrease in the rate of the endogenous metabolism was unusually steep and quite possibly was not rectilinear in shape. Also, for each of these four amino acids, other experiments in which generally better agreement between the excretions of the first and third periods were obtained, three in the case of methionine, two in the case of phenylalanine, five in the case of cystine, and one in the case of tyrosine, gave no evidence of a depression of the endogenous metabolism.

Of the twenty-two experiments reported in table 3, nine gave positive differences in urinary nitrogen excretion between the test period, during which a supplement of an amino acid mixture or a complete protein (egg) was given, and the average excretion for the initial and final periods on the basal nitrogen-free diet, although only two were of sufficient magnitude to indicate incomplete utilization. Of the thirteen negative differences, seven exceeded 4 mg., but all were obtained in experiments in which the indicated decrease in endogenous metabolism from the first to the third experimental periods was unusually great, ranging from 13.6 to 28.4 mg. of nitrogen daily. Also, for the first three amino-acid mixtures for which these large negative differences were obtained, at least three other experiments in each case, in which more favorable conditions for detecting a depression of the endogenous nitrogen prevailed, because of nearly equal urinary nitrogen excretions in periods 1 and 3, revealed no evidence of such a depression. These experiments are repetitions on the same rats of experiments yielding the suspiciously large negative differences.

Thus, the evidence as a whole does not support the proposition that the endogenous metabolism of nitrogen may be depressed by the feeding of single amino acids, of amino acid mixtures, one of which (no. 4) has been shown to support

nitrogen equilibrium in the rat, or of a complete protein mixture (egg) of proved high biological value.

Creatinine determinations were made on the urine collections of a number of these experiments. The average amounts of creatinine nitrogen in the urine daily, as well as the percentages of creatinine nitrogen on the total urinary nitrogen, for all rats receiving the same amino acid supplement and for all experimental periods, are summarized in table 4.

These results are of interest in showing a fairly consistent increase in the percentage of creatinine nitrogen in the urine

TABLE 4

Average daily excretion of creatinine nitrogen for the three experimental periods for each nitrogenous supplement used

AMINO ACID	INITIAL N FREE PERIOD		AMINO ACID PERIOD		FINAL N-FREE PERIOD	
	Total	Urinary N	Total	Urinary N	Total	Urinary N
	mg.	%	*mg.*	%	*mg.*	%
Threonine	2.95	6.02	3.16	6.94	1.60	4.24
Isoleucine	1.60	4.24	3.09	7.80	2.86	7.03
Valine	4.06	6.00	3.96	6.46	3.87	7.72
Histidine	5.80	7.40	6.24	9.50	6.03	10.68
Lysine	3.28	7.05	2.95	6.73	2.86	7.49
Phenylalanine	6.01	7.64	6.41	9.02	4.46	7.10
Cystine	3.23	5.29	3.25	5.59	3.35	6.41
Glutamic acid	2.86	7.49	3.05	7.86	2.81	8.02
Mixture no. 1	2.63	5.80	2.68	7.01	2.60	7.06
Mixture no. 2	3.20	7.08	3.18	7.45	2.99	7.93
Mixture no. 3	2.73	6.67	2.72	8.27	2.58	8.31
Average	3.49	6.40	3.70	7.51	3.27	7.45

from period 1 to period 2, suggesting that all of the deposit protein had not been catabolized in the preliminary period. The general decrease in observed daily urinary nitrogen from period 1 to period 3 may thus be largely accounted for in this way. With respect to the average amounts of creatinine nitrogen excreted daily in the various periods, there were no consistent differences, although in the averages for all rats, the excretion in period 2 exceeded those in the adjacent periods. For the various amino acids there was no consistent indication that the creatinine excretion was increased by amino acid

feeding, although it should be remembered that only small amounts were fed.

An attempt was made to maintain the food intakes of the rats constant in all three experimental periods, and generally the attempt was successful. In all experiments reported in table 3, the daily intake of basal ration was identical in the three periods. In twenty-three of the forty-three experiments reported in table 2, this ideal was also realized, and in twelve other experiments it was approximately realized. Since the excretion of endogenous nitrogen is apparently not affected by variable intakes of a nitrogen-free diet unless the intake of energy is distinctly inadequate (Mitchell, '24), it is not believed that the failures to maintain a constant intake of food throughout these experiments seriously modified the excretion of urinary nitrogen.

The excretions of nitrogen in the feces, although measured in all of these experiments, will not be reported, because they indicate complete absorption of the supplementary nitrogen fed. The average excretions of fecal nitrogen daily per gram of food consumed for all experiments were 1.63 mg. in period 1, 1.53 mg. in period 2, and 1.46 mg. in period 3.

SUMMARY AND CONCLUSIONS

Although thirteen individual amino acids, including all of those considered essential for growth, four amino-acid mixtures, including one that proved adequate for the maintenance of nitrogen equilibrium in the adult rat, and one complete protein mixture of high biological value (egg) were tested, no evidence was obtained that the endogenous metabolism could be depressed by such nitrogen-containing supplements.

The independence of the endogenous and exogenous types of nitrogen metabolism is thus confirmed.

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EFFECT OF COOKING UPON THE THIAMIN CONTENT OF FOODS

ELIZABETH AUGHEY[1] AND ESTHER PETERSON DANIEL[2]
Bureau of Home Economics, United States Department of Agriculture, Washington, D. C.

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The extent to which foods lose thiamin during cooking is not very well known, as may be seen readily by a brief survey of the tables recently compiled by Fixsen ('38). Although thiamin has long been considered relatively unstable, evidence now indicates that cooking destroys less of this vitamin than was formerly supposed.

The present experiments were conducted to determine the effect of different cooking methods on the thiamin content of a representative variety of foods. Raw and cooked foods as well as the liquid drained from the cooked products were assayed.

The meat, cereal products, dried beans, potatoes (baked), and spinach were prepared for feeding once per week; the carrots, potatoes (boiled), snap beans, and green peas were cooked twice each week. With the exception of the dried beans, cereals and potatoes, the foods were cooked immediately after reaching the laboratory. All of the foods were stored in refrigerators in tightly covered containers until used. With the exception of the carrots and spinach, which were stored at freezing temperatures during their interval of use, all of the other foods, raw and cooked, were stored in a refrigerator kept at a temperature not to exceed 5°C. All of the cooked foods were ground, cut, or mashed for feeding before refrigeration; the raw vegetables, excepting carrots and spinach, were finely chopped just prior to feeding.

[1] Transferred to National Bureau of Standards, U. S. Dept. Commerce, February 8, 1940.

[2] Transferred to Food and Drug Administration, U. S. Dept. Agriculture, November 15, 1939.

The thiamin content of the foods was determined by the rat-growth method of assay outlined by Booher and Hartzler ('39) employing crystalline thiamin hydrochloride as a standard. The basal diet designated as no. 111 consisted of casein (extracted with 60% alcohol) 18, agar 2, autoclaved yeast 15, Osborne and Mendel salts 4, cod liver oil 4, cottonseed oil 6, and cornstarch 51 parts. Before beginning an assay a few exploratory animals were given different amounts of the test food for the purpose of obtaining a rough indication of its thiamin potency.

The quantities of the test food for the assay proper were then selected with an aim to give growth rates just over and under that attained by 3 micrograms (1 International Unit) of thiamin hydrochloride. After the rats ceased growing, which required approximately 14 days, groups consisting of six to eight animals were given daily, in addition to the basal diet, the two predetermined levels of the test food and 3 micrograms of the thiamin hydrochloride, respectively.

Each animal in the group receiving thiamin had a littermate of the same sex and comparable weight in both groups receiving the test food. Animals of another group, littermates of those in the first three groups, were continued without supplement and thus served as negative controls. The quantity of supplement necessary to give exactly the same growth as 3 micrograms of the standard was finally determined by interpolation.

Preparation and sampling

Carrots. Long pointed carrots of a very deep orange color, grown in Texas and California, were purchased on the open market in Washington, D. C., at intervals from June until February. The carrots were scrubbed, dried on paper towels, scraped, and halved lengthwise. One set of halves was grated and fed raw; the other was cooked. In the pressure cooker method the halves were placed without added water in a small covered pan which was set on the rack of the cooker. After exhausting the cooker, the pressure was brought up to 15 pounds

(250°F.) as rapidly as possible (1 to 2 minutes required) and held at that pressure for a period of 11 minutes for small halves, 13 minutes for medium, and 15 minutes for large halves. In the boiling method the carrot halves were dropped into a small amount of boiling water, 100 ml. of water to 300 gm. of carrot, and boiled gently in a covered pan until tender, on the average 23 minutes. In both cases the cooked carrots were transferred to a glass bowl and chopped very fine with a two-bladed curved chopping knife.

Potatoes. Irish Cobbler potatoes, grown in Maine and selected for sizes weighing 150 to 200 gm., were boiled and baked. In the boiling method, whole pared potatoes were dropped into sufficient boiling salted water to cover them and gently boiled in an uncovered pan until tender, an average of 36 minutes being required. The cooked potatoes were mashed and thoroughly mixed. The cooking liquid was made up to such volume that 1 ml. represented 1 gm. of raw potato.

Unpared potatoes were baked at an oven temperature of 190°C. (375°F.) until soft, or an average of 63 minutes. After cooling they were removed from their skins, mashed, and thoroughly mixed. The skins were cut finely with scissors and proportionate amounts fed along with the mashed pulp.

Pared raw potatoes were grated and mixed, and weighed supplements were fed for comparison with the boiled potatoes. Unpared raw potatoes, after grating and mixing, were assayed for comparison with the thiamin content of the baked tubers.

Spinach. Bloomsdale Savoy spinach, purchased on the local market, was washed in four changes of water and then tossed on towels until visible moisture had been absorbed. Waste, consisting of roots, very coarse stems, withered or decayed leaves, and occasional bud stalks, was removed. A 500 gm. quantity of cleaned, dried, waste-free spinach was dipped in water and drained until just 100 gm. of water was left clinging to the leaves, which were then cooked with no additional water. The spinach was heated in a covered saucepan at moderate

heat for 2 minutes when the leaves began to wilt. Then the cover was removed and the spinach boiled gently for 7 minutes. The cooked spinach was drained and finely chopped before feeding. The water drained from the cooked leaves was made up to volume so that 1 ml. represented the amount from 5 gm. of uncooked spinach. Cleaned raw spinach chopped fine in a glass bowl with a food chopper was assayed for comparison with the cooked product.

Green peas. Laxton Progress peas, grown in the State of Washington, were purchased on the open market in Washington, D. C. After shelling, washing, and drying on paper towels, 500 gm. of peas were dropped into approximately 250 ml. of boiling water and simmered with occasional stirring in an uncovered vessel for 12 minutes.

Additional peas were cooked with soda; 0.3 gm. (1/16 teaspoon) of sodium bicarbonate per 500 gm. of peas was dissolved in the water before the addition of the vegetable. This method preserved only a slightly more intense green color than simmering in the absence of soda and did not affect the flavor of the peas.

Both cooked and raw peas were chopped finely before being fed. The cooking water from the peas was made up to volume so that 1 ml. represented the quantity in which 2 gm. of raw peas had been cooked. The cooking liquid from peas without soda had an average pH of 6.4; with soda the average pH was 7.4.

Snap beans. Bountiful variety snap beans, grown at the National Agricultural Research Center, Beltsville, Maryland, were used in this test. After discarding blossom and stem ends the beans were washed, dried on paper towels, broken in half, and added to sufficient boiling salted water to cover, approximately 450 ml. per 300 gm. of beans. They were boiled in a covered pan for 1 minute; the cover was then removed and boiling continued for a total of 40 minutes. The cooking liquid showed an average pH of 5.8.

Beans were also cooked in the same manner as described above except for the addition of 0.3 gm. of sodium bicarbonate

per 300 gm. of beans. This amount of soda, placed in the water before the addition of the beans, preserved green color but did not affect flavor. The cooking liquid had an average pH of 6.6.

In each case the liquid drained from the cooked beans was made up to volume for feeding so that 1 ml. represented the quantity in which 3 gm. of beans had been cooked.

Navy beans. Dried navy beans purchased on the open market were soaked for 16 hours in water (1 cup of beans to 2 cups of water). The water was drained off and the beans were dropped into boiling salted water (3 cups water to 1 cup dry beans) and were boiled gently until soft. This required an average time of 85 minutes.

Except for the addition to the cooking water of 0.4 gm. of sodium bicarbonate (a small "pinch") per cup of dry beans, the same method was used for cooking beans with soda. This amount of soda reduced the cooking time approximately one-third.

The cooked beans were mashed to a thick paste with the remaining small amount of cooking liquid for supplement feeding. The raw dry beans were ground in a food grinder until practically pulverized before feeding.

The liquid drained from the beans which had soaked overnight had an average pH of 6.5. Liquid from the beans cooked without soda had a pH of 5.7; liquid from those cooked with soda showed a pH of 6.0.

Rolled oats. Commercially packaged rolled oats, purchased in Washington, D. C., were used. One cup of the oats was added to 2 cups of boiling salted water and stirred and cooked for 2 minutes over an open flame. Cooking was continued in a double boiler for a total of 2 hours.

Whole wheat. A sample of hard red spring wheat grown near Great Falls, Montana, was cooked both as a cereal and as bread. For use as a cereal 1 cup of wheat, after being cracked, was added to 2½ cups of boiling salted water, boiled and stirred for 2 minutes over an open flame, and finally cooked for an additional 28 minutes in a double boiler.

To test the effect of baking on the thiamin content of wheat, part of the whole wheat was ground into flour and a water bread containing no other flour was prepared. The bread was baked for a total period of 45 minutes; the baking temperature for the first 15 minutes was 218°C. (425°F.); for the remaining 30 minutes the temperature was reduced to 190°C. (375°F.). After baking, the loaf was sliced, dried at a moderate temperature (approximately 45°C.), and ground to fine crumbs for feeding.

Pork. Eight-rib sections of pork loin were purchased weekly on the open market, trimmed, and one thick chop with rib bone was cut from each end. The six-rib section remaining was prepared as a roast. The two chops were used to estimate the relative proportion of lean in the whole piece in order to relate raw and cooked weights. The lean from these two chops was ground twice, and used for the raw pork assay.

The loin roasts were cooked at an oven temperature of 175°C. (350°F.) until the internal temperature of the roasts reached 84°C. (183°F.), and the lean portion removed and ground twice for the assay.

The drippings from the roast, washed from the pan with small quantities of water, were chilled, freed from fat, made up to volume, and fed.

Subsequently another weekly series of four-rib sections of pork loin were purchased. After determining the relative proportion of lean in two of the chops as in the above experiment, the lean portion was ground and fed raw. The remaining two chops were braised, i.e., browned on each side in an uncovered skillet, and the cooking continued with the skillet covered, until the meat was well done. The lean meat from the braised chops was ground twice and fed.

RESULTS AND DISCUSSION

No loss of thiamin was observed in carrots prepared either by the boiling or pressure cooker methods. This is contrary to the findings of Richardson and Mayfield ('32) who reported a 20% loss of vitamin B_1 in cooking (boiling) carrots. These

same investigators found that stored potatoes also suffered a 20% loss of thiamin during boiling.

Baker and Wright ('35) found that potatoes boiled in their skins contained 0.3 International Units per gram as compared with 0.4 International Units per gram in samples of raw potatoes. The present studies indicated a 33% loss of thiamin in the boiled pared potato. Twenty per cent of this had been destroyed while about 13% had been dissolved in the cooking liquid. Baking caused 16% destruction of thiamin in potatoes.

During cooking the spinach lost 30% of its thiamin value; 22% of the total thiamin content was destroyed and 8% was recovered in the cooking liquid. Roscoe ('30) found that spinach boiled 15 minutes lost about half of its vitamin B_1 content in the cooking liquid. Hoff ('33) reported more than 50% loss of vitamin B_1 in spinach cooked by "ordinary household methods." Excessive vitamin B_1 losses reported by some investigators may have been the result of cooking in too large quantities of water or of employing too long a cooking time.

Green peas simmered without the use of soda retained 80% of their thiamin; peas simmered with a small amount of soda retained only 67% of this vitamin. In both cases 11% of the thiamin was dissolved in the cooking liquid. There was a 22% destruction of the vitamin in the presence of sodium bicarbonate, more than twice as much as was destroyed when no soda was used. Baker and Wright ('38) have reported thiamin values for fresh peas ranging from 1.6 to 2.8 International Units per gram; those of cooked fresh peas 0.8 to 1.2 International Units. Rose and Phipard ('37) found a 26% loss of vitamin B_1 in cooking peas for 15 minutes.

Snap beans cooked without soda retained about 68% of their original thiamin content. Eighteen per cent was destroyed and 14% dissolved in the cooking liquid. Approximately 60% of the thiamin in snap beans cooked with a "pinch" of soda was destroyed. About 40% was retained by the beans; only a slight trace was found in the cooking

liquid. Mayfield and Richardson ('39) found no loss in thiamin value after cooking snap beans 45 minutes. They found 0.30 International Units of thiamin per gram in raw beans, 0.33 International Units per gram in the cooked beans.

Navy beans cooked in tap water with or without soda retained all of their original thiamin value. Soda shortened the time of cooking by a little more than one-third, or approximately 30 minutes. The effect of cooking with soda for 30 minutes longer was not determined inasmuch as this would have no practical value. Lantz ('38) found about 50% loss in the thiamin content of dried pinto beans soaked 16 hours and cooked 2¾ hours in distilled water. This loss was greater when tap water was used or when a small amount of sodium bicarbonate was added. Part of the loss reported by this investigator may be accounted for by the fact that the cooked beans were dried, ground, and stored before testing. Mickelsen, Waisman and Elvehjem ('39) found that drying (animal tissues) under vacuum at temperatures above 70°C. caused appreciable destruction of vitamin B_1.

The preparation of whole grain wheat or oat cereals in the manner described caused no destruction of the thiamin content. Wheat prepared as bread, however, lost about 15% of its thiamin. This comparatively small loss is in agreement with the findings of Copping and Roscoe ('37), Morgan and Frederick ('35), and Scheunert and Schieblich ('37), all of whom report very little or substantially no destruction in the thiamin value of the original flours in the baking of bread.

Pork cooked as a roast lost 43% of its thiamin value. Only a trace of thiamin was recovered in the drippings. Braising caused about 15% destruction of this vitamin. Christensen, Latzke and Hopper ('36) reported a 12% destruction of vitamin B_1 in pork which had been ground and subsequently stirred and cooked in a double boiler until it reached a constant temperature of 90°C. This was dried and ground for assay. Mickelsen, Waisman, and Elvehjem ('39) found a thiamin destruction of 50% caused by roasting pork loin and a 35% loss due to frying. This is comparable to our findings

TABLE 1

Thiamin in raw and cooked foods and losses during cooking

FOOD	COOKING METHOD	TIME OF COOKING	AVERAGE COOKED WT. OF 100 GM. RAW FOOD	THIAMIN CONTENT					
				Per 100 gm. cooked food	Per 100 gm. raw food	In cooked food per 100 gm. raw wt.	Retained by cooked food	Dissolved in cooking liquid	Destroyed during cooking
		min.	*gm.*	*I.U.*	*I.U.*	*I.U.*	%	%	%
Carrots [1]	Pressure cooker	11–15	90	20	19	18	100	—	0
	Boiled	23±3 [2]	95	25	22	24	100	0	0
Potatoes	Baked	63±5 [2]	85	50	50	42	84	—	16
	Boiled (pared)	36±5 [2]	95	32	45	30	67	13	20
Spinach	Boiled	9	80	38	43	30	70	8	22
Peas, green	Simmered	12	97	124	150	120	80	11	9
	" with soda	12	99	100	150	100	67	11	22
Beans, snap	Boiled	40	85	18	22	15	68	14	18
	" with soda	40	85	11	22	9	41	Trace	59
Beans, navy	Boiled	83±22 [2]	320	75	250	240	100	—	0
	" with soda	53±4 [2]	330	80	250	265	100	—	0
Oats, rolled	Double boiler	120	635	33	200	210	100	—	0
Wheat, whole	Double boiler	30	485	36	180	175	100	—	0
	Baked (bread)	45	155	100	180	155	86	—	14
Pork loin (lean portion)	Braised (chop)	13±1 [2]	75	550	485	413	85	—	15
	Roasted	43±3 [2] per lb	65	400	455	260	57	Trace	43

[1] The assays on carrots were conducted under the direction of Dr. Hazel E. Munsell.

[2] Average deviation.

for roasting but their frying method appears to have caused an appreciably greater destruction of thiamin than the braising method used in these experiments.

The findings of the present experiments are summarized in table 1. Some interesting practical facts come to light in an analysis of these data. The whole-grain cereals and dried legumes included in every list of thiamin-rich foods furnish less of this vitamin to the diet than is generally supposed. In the past these lists have been made up on the basis of raw foods and the large proportion of water absorbed during preparation leaves an average serving of the cooked product with a relatively lower thiamin value than is ordinarily realized. On a serving basis baked potatoes, including skin, rank higher in thiamin than cooked whole wheat or oat cereals. Even boiled potatoes, spinach, and carrots may be classed as furnishing amounts of thiamin per serving comparable to those supplied by these cooked cereals. One slice of an all-whole-wheat bread also contains approximately the same quantity of thiamin as one serving of these vegetable and cereal foods. A rough estimate of ten servings of these foods would be needed to meet the liberal daily allowances often recommended (Cowgill, '38). Navy beans, taking up less water during cooking, furnish proportionately more thiamin per serving than the cereals.

The seeds in snap beans as they are usually eaten are too immature to make this vegetable a rich source of thiamin. Green peas, on the other hand, supply in a single serving about one-fourth to one-third of the quantity of thiamin usually recommended for a liberal daily allowance.

In contrast to the other foods which were analyzed, one serving of the lean portion of pork loin cooked either as chop or roast furnishes this plentiful daily allowance of thiamin for the adult.

SUMMARY

Thiamin losses due to different cooking procedures were determined by the rat-growth method for a representative variety of foods. Experiments were set up to show the per-

centage of the thiamin originally present in the raw food that was (1) retained by the cooked product, (2) dissolved in the cooking water, and (3) destroyed.

Thiamin destruction amounted to as much as 22% in some vegetables boiled in water and additional amounts up to 15% dissolved in the cooking water. In cases where the cooking water is discarded total thiamin losses in vegetables may amount to approximately 20 to 35%. The addition of a small amount of sodium bicarbonate markedly increased the destruction of thiamin in green peas and snap beans but had no significant effect upon the thiamin content of boiled navy beans. Roasting caused a loss of 43% of the thiamin in pork loin, nearly three times as much destruction as braising. Double boiler cooking of whole grain cereals did not destroy thiamin; baking bread caused about 15% loss of this vitamin. The relative values of the different cooked foods in meeting the daily human requirements for thiamin are discussed.

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THE METABOLISM OF TYROSINE, ASPARTIC ACID AND ASPARAGINE, WITH SPECIAL REFERENCE TO RESPIRATORY EXCHANGE AND HEAT PRODUCTION [1]

MAX KRISS AND LAWSON F. MARCY
Institute of Animal Nutrition, The Pennsylvania State College, State College

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In a previous paper (Kriss, '39) determinations of the metabolizability of glutamic acid, glycine and alanine were reported, based on balances of nitrogen, carbon and energy, and the relationships between the urinary nitrogen, respiratory exchange and metabolizable energy for each of these amino acids—factors usable in indirect calorimetry for the computation of heat production—were determined. These factors were found to be closely correlated with the ratios of carbon to nitrogen in the different amino acids. It seemed desirable to extend the study to other amino acids; and the present paper presents the results obtained with the amino acids tyrosine, aspartic acid and asparagine.

EXPERIMENTAL

The experimental procedure employed in this investigation was, in most respects, the same as that followed in the previous study (Kriss, '39). Sixteen male albino rats weighing approximately 200 gm. each served as the experimental subjects. The animals were first placed on a basal ration of 8 gm. per day, this ration consisting of 93.7% of an approximately complete calf meal (Forbes, Kriss and Miller, '34) and 6.3% of butterfat. This diet was adequate for maintaining

[1] Authorized for publication on October 9, 1939, as paper no. 925 in the journal series of the Pennsylvania Agricultural Experiment Station.

the animals in approximate equilibrium of nitrogen and energy. The animals were kept on the ration for periods of from 2 to 3 weeks, and feces and urine were collected from each rat during the final 7-day period.

Following this treatment six of the animals received in addition to the basal ration 1.288 gm. of l-tyrosine per day; five received 2 gm. of l-aspartic acid, and five received 2 gm. of l-asparagine as the daily supplements. These supplemented diets were fed to the rats for a period of 8 days. Feces and urine were collected during the last 5 days.

During the entire experimental period the food was offered the animals in equal portions twice a day, as in the previous studies.

TABLE 1

Analysis of basal ration and of amino acid supplements

MATERIAL	MOISTURE	NITROGEN	CARBON	ENERGY
	%	%	%	*Calories per gram*
Basal ration	10.38	3.25	42.26	4.306
l-tyrosine	0.29	7.62	58.74	5.824
l-aspartic acid	0.05	10.34	36.19	2.888
l-asparagine	12.03	18.28	31.97	3.011

The basal ration and the amino acid supplements [2] were analyzed for nitrogen, carbon, energy and moisture. The urine and feces were analyzed for energy, carbon and nitrogen. Energy was determined in the urine composited for each group of animals, while nitrogen and carbon were determined in the urine of each individual rat. The feces were composited for each group before being analyzed. As in the previous experiments, the excretory products representing the amino acid supplements were determined as the differences between the residues from the basal ration and the supplemented diets.

RESULTS

The analyses of the basal ration and of the amino acids used as supplements are presented in table 1. The large

[2] Purchased from Eastman Kodak Co., Rochester, N. Y.

percentage of moisture in the asparagine corresponds almost exactly to 1 molecule of water of crystallization. The percentages of nitrogen and carbon in all three amino acids, expressed on the dry matter basis, differ by less than 2% from the theoretical values.

The results of the urinary analysis, presented in table 2, show in a consistent and significant manner, as in the previous study, the influence of the amino acid feeding on the nitrogen and carbon content of the urine and, especially, on the C : N and energy : N ratios. The average ratio of carbon to nitrogen in the urine from the basal ration is 0.77 : 1. Supplementing the basal diet by 1.288 gm. of tyrosine raised the ratio of carbon to nitrogen in the urine to 1.61 : 1. On the other hand, the addition to the basal diet of 2 gm. of either aspartic acid or asparagine had the effect of slightly lowering the C : N ratios of the urine.

The urinary C : N ratios representing aspartic acid (0.50 : 1) and asparagine (0.45 : 1) are only slightly higher than the C : N ratios of urea (0.43 : 1). Similarly, the urinary energy : nitrogen ratios for aspartic acid (6.30 : 1) and for asparagine (5.80 : 1) are only slightly higher than the ratio of energy to nitrogen found in urea (5.43 : 1) (Kriss and Marcy, '40). However, tyrosine is represented by ratios of carbon to nitrogen (2.77 : 1) and energy to nitrogen (25.18 : 1), in the urine, which are several times as high as the corresponding ratios existing in urea. These C : N and calories : N ratios indicate that nearly all of the urinary nitrogen derived from aspartic acid or asparagine represents metabolized amino acid, and that a relatively large proportion of tyrosine is eliminated in the urine in unmetabolized or incompletely metabolized form. A significant fact in this connection is that the urine from the tyrosine-supplemented ration was nearly black, indicating the presence of homogentisic acid.

We have not found it convenient during the experiments to determine directly the urea nitrogen and the amino acid nitrogen in the urine. We have, therefore, followed the method outlined in the previous publication (Kriss, '39) of estimat-

TABLE 2

Urinary analysis

RAT NO.	BASAL RATION				BASAL RATION PLUS 1.288 GM. TYROSINE				DIFFERENCES DUE TO AMINO ACID				
	Nitrogen per day	Carbon per day	C : N ratio	Energy per day	Nitrogen per day	Carbon per day	C : N ratio	Energy per day	Nitrogen	Carbon	C : N ratio	Energy	Energy : N ratio
	mg.	*mg.*		*cal.*	*mg.*	*mg.*		*cal.*	*mg.*	*mg.*		*cal.*	
41	134	114	0.85		264	428	1.62		130	314	2.42		
42	153	117	0.77		276	428	1.55		123	311	2.53		
43	146	111	0.76		266	442	1.66		120	331	2.76		
44	168	128	0.76		264	438	1.66		96	310	3.23		
45	165	127	0.77		274	418	1.53		109	291	2.67		
46	153	110	0.72		256	418	1.63		103	308	2.99		
Average	153	118	0.77	1256	267	429	1.61	4126	114	311	2.77	2870	25.18
					BASAL RATION PLUS 2 GM. ASPARTIC ACID								
51	156	134	0.86		327	218	0.67		171	84	0.49		
52	187	145	0.78		316	218	0.69		129	73	0.57		
53	194	141	0.74		296	193	0.65		102	52	0.51		
54	168	122	0.73		337	192	0.57		169	70	0.41		
55	143	114	0.80		298	199	0.67		155	85	0.55		
Average	170	131	0.77	1387	315	204	0.65	2301	145	73	0.50	914	6.30
					BASAL RATION PLUS 2 GM. ASPARAGINE								
56	138	110	0.80		434	243	0.56		296	130	0.44		
57	157	120	0.76		460	265	0.58		303	145	0.48		
58	158	122	0.77		483	265	0.55		325	143	0.44		
59	175	129	0.74		490	267	0.55		315	138	0.44		
60	178	129	0.73		460	255	0.55		282	126	0.45		
Average	161	122	0.76	1279	465	259	0.56	3041	304	136	0.45	1762	5.80

ing the portions of the urinary nitrogen which represent metabolized and unmetabolized amino acid, respectively (table 3), on the basis of the C : N and energy : N ratios. There is evidence (table 5), which will be discussed later, to show that under the existing experimental conditions, this method of partitioning the urinary nitrogen yields correct values for the quantities of amino acid metabolized.

TABLE 3

Metabolizability of amino acids

	ASPARTIC ACID 2.0 GM.		ASPARAGINE 2.0 GM.		TYROSINE 1.288 GM.
Income					
Energy, calories	5776		6022		7501
Carbon, mg.	724		639		757
Nitrogen, mg.	207		366		98
Outgo					
Feces					
Energy, calories	389		318		349
Carbon, mg.	35		28		21
Nitrogen, mg.	10		12		3
Urine	AS DETERMINED	CORRECTED TO N-EQUILIBRIUM	AS DETERMINED	CORRECTED TO N-EQUILIBRIUM	CORRECTED TO N-EQUILIBRIUM
Energy, calories	914	1242	1762	2052	2714
Carbon, mg.	73	99	136	158	296
Total nitrogen, mg.	145	197	304	354	95
Unmetabolized amino acid N, mg.[1]	4	5	5	6	35
Unmetabolized amino acid N, mg.[2]	6	8	10	12	31
Metabolized amino acid N, mg.[1]	141	192	299	348	60
Metabolized amino acid N, mg.[2]	139	189	294	342	63
Metabolized amino acid N, %[1]	97	97	98	98	63
Metabolized amino acid N, %[2]	96	96	97	97	66
Metabolizable					
Energy, calories	4473	4145	3942	3652	4438
Carbon, mg.	616	590	475	453	440
Energy, %	77.4	71.8	65.5	60.6	59.2
Carbon, %	85.1	81.5	74.3	70.9	58.1

[1] Computed on the basis of C: N ratio.

[2] Computed on the basis of energy: N ratio.

The balances of nitrogen, carbon and energy for the different amino acids are presented in table 3. The data for feces and urine are the average values for the respective groups of animals. The data for total urinary nitrogen, carbon and energy representing aspartic acid and asparagine, and consequently, the values for metabolizable energy and carbon of these amino acids are given on two bases: (1) as determined and (2) corrected to represent nitrogen equilibrium. The corrections of the energy and carbon of the urine to nitrogen equilibrium were based on the relationships, as directly determined, between the urinary energy and nitrogen and between the urinary carbon and nitrogen, respectively. In other words, the determined values for urinary energy and carbon were increased in the same proportion as the nitrogen of the urine had to be increased to represent nitrogen equilibrium. The directly determined balances of nitrogen indicate that of the total nitrogen ingested as aspartic acid and as asparagine 25.1% and 13.7%, respectively, were retained in the body.

In the case of tyrosine feeding, however, the increase in elimination of urinary nitrogen (114 mg.) caused by this supplement (see table 2) exceeded somewhat the nitrogen content of the latter (98 mg.), thus indicating that none of the tyrosine was retained in the body. In order to compute the relationship between the tyrosine ingested and the products eliminated, the data for urinary nitrogen, carbon and energy for this supplement were corrected, as in table 3, to a basis of nitrogen equilibrium, this correction being made on the assumption that the excess of nitrogen eliminated in the urine during the period of feeding the tyrosine-supplemented diet was derived from the protein of the basal ration rather than from body protein, inasmuch as the total nitrogen balance during this period was slightly positive (+15 mg.).

In giving the foregoing interpretations the writers are not unaware of the recent study of tyrosine metabolism by Schoenheimer, Ratner and Rittenberg ('39), who used isotopic nitro-

gen, and found that a considerable part of the labeled nitrogen of dietary tyrosine was transferred to and deposited in the tissue proteins. The finding that samples of pure tyrosine isolated from liver proteins and from body proteins contained a high concentration of labeled nitrogen was rightly considered by the authors as indicating that some dietary tyrosine was directly deposited in the tissue proteins. They have also observed that while the nitrogen of the dietary tyrosine was only partly excreted in the urine, an equivalent amount of protein nitrogen was excreted, thus effecting nitrogen equilibrium.

These observations of Schoenheimer and co-workers are interesting from the point of view of intermediary metabolism, and serve to emphasize the fact that balances of nitrogen do not reveal the intimate character of the intermediary reactions.

Insofar as the results of the present study are concerned, it should be borne in mind that they deal chiefly with the ultimate effects as shown by the balances of nitrogen, carbon and energy, and that the interpretations of these results are presented largely from this point of view.

The results of partitioning the urinary nitrogen (table 3) into fractions representing metabolized and unmetabolized amino acid show, in accord with the C : N ratios and energy : N ratios of the urine, that tyrosine was much less completely metabolized than was either aspartic acid or asparagine. Calculated on the basis of C : N ratios, the percentages of the total nitrogen excreted in the urine representing metabolized amino acid are 63 for tyrosine, 97 for aspartic acid, and 98 for asparagine. Values close to these were obtained when the calculation was based on the energy : N ratios.

Of the total energy ingested as tyrosine 59.2% was metabolizable. The metabolizable energy values of aspartic acid and of asparagine (corrected to a basis of nitrogen equilibrium) were found to be 71.8% and 60.6%, respectively, of the gross energy of the amino acids.

The carbon was found to be metabolizable (on a N-equilibrium basis) to the extent of 58.1% for tyrosine, 81.5% for aspartic acid, and 70.9% for asparagine.

The relatively low metabolizability of the carbon and energy of asparagine, as compared with aspartic acid, was associated with the greater nitrogen content of the former. The low metabolizability of the carbon and energy of tyrosine was apparently due to the excretion in the urine of a relatively large proportion of this amino acid in unmetabolized form.

Table 4 sets forth the computations of the respiration and energy factors for the different amino acids. Attention is called to the facts that in this table, as in the corresponding table of the previous publication (Kriss, '39), the data representing the intake are on the basis of pure amino acids, and that the data for urinary constituents and, consequently, for metabolizable products are on the basis of nitrogen equilibrium.

The values for carbon and energy of the urine were computed in relation to the urinary nitrogen, on the basis of the data given in table 3. The values for fecal nitrogen, carbon and energy were computed in proportion to the nitrogen intake from the data of table 3. The values for hydrogen and oxygen of the feces were computed from the fecal nitrogen on the assumption that the latter represents the unabsorbed portion of the amino acid. The values for hydrogen and oxygen of the urine were computed from the calculated values of urea and unmetabolized amino acid present. The important relationships derived in table 4 are given in the lower part of the table.

The values for O_2, CO_2 and calories per gram of urinary nitrogen are approximately twice as great for aspartic acid as the corresponding values for asparagine. The same factors for tyrosine are approximately four times as great as those for asparagine. As in the previous study with other amino acids, these factors show a close correlation with the ratios of carbon to nitrogen in the materials, these ratios being,

TABLE 4

Determination of factors for computing the respiratory exchange and the heat production in the metabolism of tyrosine, aspartic acid and asparagine

	TYROSINE	ASPARTIC ACID	ASPARAGINE
Intake, computed per 100 gm. of pure amino acids			
Energy, Calories	584.1	288.9	342.3
Nitrogen, gm.	7.73	10.52	21.20
Carbon, gm.	59.67	36.07	36.34
Hydrogen, gm.	6.08	5.26	6.06
Oxygen, gm.	26.52	48.10	36.34
Outgo			
Feces			
Energy, Calories	27.9	19.8	18.4
Nitrogen, gm.	0.24	0.51	0.70
Carbon, gm.	1.66	1.78	1.62
Hydrogen, gm.	0.19	0.26	0.20
Oxygen, gm.	0.82	2.33	1.20
Urine			
Energy, Calories	214.0	63.1	118.8
Total nitrogen, gm.	7.49	10.01	20.50
Unmetabolized amino acid N	2.60	0.35	0.51
Metabolized (urea) N, gm.	4.89	9.66	19.99
Carbon, gm.	23.34	5.04	9.17
Hydrogen, gm.	2.75	1.56	3.01
Oxygen, gm.	11.71	7.12	12.39
Metabolized			
Energy, Calories	342.2	206.0	205.1
Carbon, gm.	34.67	29.25	25.55
Hydrogen, gm.	3.14	3.44	2.85
Oxygen, gm.	13.99	38.65	22.75
Intramolecular H_2O			
Hydrogen, gm.	1.75	3.44	2.84
Oxygen, gm.	13.99	27.52	22.75
Carbon oxidized to CO_2, gm.	34.67	29.25	25.55
Hydrogen oxidized to H_2O, gm.	1.39	0.00	0.01
CO_2 produced, gm.	127.12	107.25	93.68
O_2 required to oxidize C, gm.	92.45	78.00	68.13
O_2 required to oxidize H, gm.	11.12	0.00	0.08
Intramolecular O_2 in excess of that required to oxidize H, gm.		11.13	
Total O_2 required, gm.	103.57	66.87	68.21
Respiratory quotient	0.89	1.17	1.00
Liters O_2 required per gm. urinary N	9.68	4.68	2.33
Liters CO_2 produced per gm. urinary N	8.64	5.46	2.33
Calories metabolized per gm. urinary N	45.7	20.6	10.0
Calories per liter O_2	4.72	4.40	4.30
Liters O_2 required per gm. N of amino acid metabolized	14.82	4.84	2.39
Liters CO_2 produced per gm. N of amino acid metabolized	13.24	5.66	2.39
Calories metabolized per gm. N of amino acid metabolized	70.0	21.3	10.3

for tyrosine 7.72 : 1, for aspartic acid 3.43, and, for asparagine 1.71.

The respiration and energy factors for tyrosine, expressed per gram of nitrogen of amino acid metabolized, are considerably higher than the corresponding values expressed per gram of total urinary nitrogen. With the other two amino acids the respective differences between the two sets of values are very slight. This is a result of the calculation (table 3) that only very small quantities of unmetabolized aspartic acid or asparagine appeared in the urine, and that a relatively large quantity of tyrosine was excreted in the urine in the unmetabolized form.

It is obvious from the foregoing results that the total urinary nitrogen is not always a true index of the metabolism of amino acids, and that the respiration and energy factors expressed per gram of nitrogen of amino acid metabolized can be applied with safety only when the quantity of completely metabolized amino acid is definitely known. The determined factors expressed per gram of total urinary nitrogen apply correctly to the experimental conditions. The applicability of these factors to other conditions of experimentation would depend on the constancy of the unmetabolized fraction of any particular amino acid appearing in the urine. It is noteworthy that in previous experiments with rats the levels of intake of heart muscle protein, casein and gelatin (Kriss and Voris, '37) and of glycine (Kriss, '39) had but a slight effect on the respiration and energy values per gram of urinary nitrogen; and that the proportions of glycine, alanine and glutamic acid, respectively, found by us to be excreted in unmetabolized form in the urine of rats are in substantial agreement with those found by Lusk ('12–'13) in the dog.

Table 5 presents a comparison between the determined and the theoretical values for the gaseous exchange and heat production per gram of nitrogen of amino acid metabolized. This table includes results obtained with other amino acids in the previous study.

The determined values are those derived in table 4, which involved the use of the computed fractions of urinary nitrogen representing amino acid metabolized, the latter fractions being the average values as calculated on the basis of C : N and energy : N ratios. The theoretical values represent the gaseous exchange and the heat of complete combustion of the amino acids minus the corresponding values of urea that can be calculated to form from them, in relation to their nitrogen content.

TABLE 5

The determined as compared with theoretical values for the gaseous exchange and heat production per gram of nitrogen of amino acid metabolized

AMINO ACIDS	HEAT PRODUCED PER GRAM N OF AMINO ACID METABOLIZED		CO_2 PRODUCED PER GRAM N OF AMINO ACID METABOLIZED		O_2 REQUIRED PER GRAM N OF AMINO ACID METABOLIZED	
	Determined	Theoretical	Determined	Theoretical	Determined	Theoretical
	Calories	*Calories*	*Liters*	*Liters*	*Liters*	*Liters*
Glutamic acid [1]	32.1	32.9	7.05	7.20	7.08	7.22
Glycine [1]	11.2	11.2	2.40	2.40	2.42	2.38
Alanine [1]	22.7	22.3	3.95	4.00	4.78	4.81
Tyrosine	70.0	70.1	13.24	13.62	14.82	15.21
Aspartic acid	21.3	22.0	5.66	5.60	4.84	4.80
Asparagine	10.3	10.7	2.39	2.40	2.39	2.40

[1] Reported previously (Kriss, '39).

The agreement between the determined and the theoretical values of table 5 is, indeed, very close for all items and for all amino acids, and cannot be considered as fortuitous. In the majority of the comparisons the differences are less than 2% of the theoretical values, and in only two cases do they exceed 3%. Of especial significance is the very close agreement between the values compared for alanine and for tyrosine, inasmuch as considerable quantities of these substances were calculated to be excreted in the urine in the unmetabolized form (table 3). Any large error in the use of the ratios of carbon to nitrogen and energy to nitrogen, especially in the case of alanine and tyrosine, would have shown up in these comparisons.

Insofar as the degree of agreement between the determined and the theoretical values depends on the fractions of urinary nitrogen calculated to represent metabolized amino acid, the very close agreement obtained indicates that the calculation of these fractions possesses a high degree of accuracy. It also indicates that the quantitative relationships between the nitrogen of the urine and its carbon and energy content, respectively, as determined for the amino acids by direct comparison of the urinary constituents from the basal ration with those from the amino acid-supplemented rations (table 2), correctly represent the amino acid supplements, as far as the ultimate metabolic effects are concerned.

SUMMARY

Tyrosine, aspartic acid and asparagine fed to rats as supplements to a mixed maintenance ration, in quantities supplying 7.5, 5.8 and 6.0 kilogram-calories per day, respectively, appeared to be absorbed to the extent of 97%, 95% and 97%. The balances of nitrogen indicated that of the total nitrogen ingested as aspartic acid and as asparagine 25.1% and 13.7%, respectively, were retained in the body, and that none of the tyrosine was retained. These balances were presumably conditioned by the composition of the basal ration.

The C : N ratios of the urinary constituents from the different supplements were as follows: from tyrosine 2.77 : 1; from aspartic acid 0.50 : 1; and from asparagine 0.45 : 1. The energy : N ratios of the urine derived from the supplements were as follows: from tyrosine 25.18 : 1; from aspartic acid 6.30 : 1; and from asparagine 5.80 : 1. On the basis of these ratios the calculated fractions of the urinary nitrogen representing metabolized amino acids were approximately, for tyrosine 65%, for aspartic acid 97% and for asparagine 98%. The metabolizable energy values of tyrosine, aspartic acid and asparagine (corrected to a basis of nitrogen equilibrium) were found to be 59.2%, 71.8% and 60.6%, respectively, of the gross energy of the amino acids.

Factors were determined for computing the respiratory exchange and the heat production in the metabolism of each of the amino acids tested. The values obtained for O_2, CO_2 and calories per gram of urinary nitrogen are approximately twice as great for aspartic acid as the corresponding values for asparagine; and the values for tyrosine are approximately four times as great as those for asparagine, thus showing, in confirmation of a previous report, a close correlation between these factors and the ratios of carbon to nitrogen in the materials.

A very close agreement was obtained between the determined respiration and energy factors, expressed per gram of nitrogen of amino acid metabolized, and the values computed on a theoretical basis.

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